Journal of Food Protection, Vol. 75, No. 4, 2012, Pages 631–636
doi:10.4315/0362-028X.JFP-11-339

Rapid and Sensitive Detection of *Salmonella* Typhimurium on Eggshells by Using Wireless Biosensors

YATING CHAI,[1]* SUIQIONG LI,[1] SHIN HORIKAWA,[1] MI-KYUNG PARK,[1] VITALY VODYANOY,[2] AND BRYAN A. CHIN[1]

[1]*Materials Research and Education Center and* [2]*Department of Anatomy, Physiology and Pharmacology, Auburn University, Auburn, Alabama 36849, USA*

MS 11-339: Received 8 July 2011/Accepted 6 December 2011

ABSTRACT

This article presents rapid, sensitive, direct detection of *Salmonella* Typhimurium on eggshells by using wireless magnetoelastic (ME) biosensors. The biosensor consists of a freestanding, strip-shaped ME resonator as the signal transducer and the E2 phage as the biomolecular recognition element that selectively binds with *Salmonella* Typhimurium. This ME biosensor is a type of mass-sensitive biosensor that can be wirelessly actuated into mechanical resonance by an externally applied time-varying magnetic field. When the biosensor binds with *Salmonella* Typhimurium, the mass of the sensor increases, resulting in a decrease in the sensor's resonant frequency. Multiple E2 phage–coated biosensors (measurement sensors) were placed on eggshells spiked with *Salmonella* Typhimurium of various concentrations (1.6 to 1.6×10^7 CFU/cm^2). Control sensors without phage were also used to compensate for environmental effects and nonspecific binding. After 20 min in a humidity-controlled chamber (95%) to allow binding of the bacteria to the sensors to occur, the resonant frequency of the sensors was wirelessly measured and compared with their initial resonant frequency. The resonant frequency change of the measurement sensors was found to be statistically different from that of the control sensors down to 1.6×10^2 CFU/cm^2, the detection limit for this work. In addition, scanning electron microscopy imaging verified that the measured resonant frequency changes were directly related to the number of bound cells on the sensor surface. The total assay time of the presented methodology was approximately 30 min, facilitating rapid detection of *Salmonella* Typhimurium without any preceding sampling procedures.

The recent *Salmonella* outbreak in eggs gained the attention of mainstream America in July 2010. The outbreak led to the recall of 380 million eggs nationwide, after the Centers for Disease Control and Prevention received a 300% increase in reported *Salmonella* cases *(19)*. One of the possible sources of the *Salmonella* is the external contamination of eggshells. *Salmonella* contamination through eggshells accounts for approximately 50% of foodborne *Salmonella* outbreaks in the United States *(14, 19)*. Raw eggs are believed to be a potentially hazardous food, and refrigeration during storage and transport is required by the U.S. Food and Drug Administration. Although the transmission mechanism for *Salmonella* contamination of eggshells has not been fully understood, feces contamination and contact of the eggshells with contaminated surfaces are possible sources leading to *Salmonella* contamination of the external surfaces of the eggshells *(20)*.

The current microbiological detection techniques, including enzyme-linked immunosorbent assay *(10)*, PCR *(8)*, Fourier transform infrared spectroscopy *(23)*, and the recently developed desorption electrospray ionization *(21)*, are time-consuming and labor intensive. Compared with these methods, the phage-based magnetoelastic (ME) biosensor used in this investigation is rapid, label-free, and inexpensive *(6, 11)*. In addition, phage-based sensors are environmentally more robust than antibody-based sensors, making the ME biosensor more stable in different detection environments and easier to store *(4, 18)*.

Recently, sensors based on magnetic properties have been widely used in the chemical and biological fields *(11, 22)*. Examples include superconducting quantum interference sensors, giant magnetic resistance sensors, microcantilevers, and flux-gate magnetometers *(21)*. Compared with these sensors, the principal advantage of the ME biosensor is wireless signal transduction *(3)*. In other words, there is no physical connection between the sensors and the signal testing system. Hence, the ME sensors can be placed directly on food surface for the detection of bacterial surface contamination. Unlike other biosensors, which require sample preparation prior to the testing (i.e., capturing, purification, and concentration), the wireless ME biosensor eliminates the preceding procedures and simplifies the testing methodology. In addition, with a portable test system, the rapid, handheld, inexpensive detection can be realized by the wireless ME biosensors.

This article presents an investigation into the rapid, direct detection of *Salmonella enterica* serovar Typhimurium on eggshells by using phage-based ME biosensors. The ME biosensor is composed of a freestanding, strip-shaped

* Author for correspondence. Tel: 334-844-3126; Fax: 334-844-3400; E-mail: yzc0012@tigermail.auburn.edu.

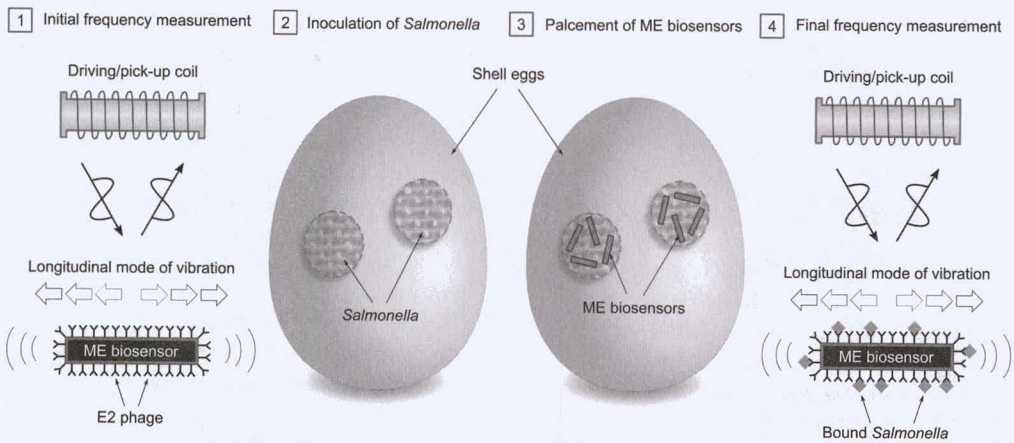

FIGURE 1. *Schematic of the process used for the detection on eggshell surface by using ME biosensors.*

ME resonator coated with a phage biorecognition layer. The ME resonator is made from an amorphous, ferromagnetic alloy that elongates or contracts when subjected to a magnetic field *(2, 9)*. In a time-varying magnetic field, the resonator converts the magnetic energy into mechanical oscillation. The resonant frequency of the resonator is related to its shape, dimensions, and materials properties. For a freestanding, strip-shaped resonator, the thickness of which is much smaller than the length and width, the fundamental resonant frequency under longitudinal vibration is expressed as *(3, 12)*:

$$f = \frac{1}{2L}\sqrt{\frac{E}{\rho(1 - v)}} \quad (1)$$

where E, ρ, and v are the elastic modulus, density, and Poisson's ratio of the material, respectively. When the ME sensor encounters target bacteria, the bacteria are captured and bound to the sensor's surface through the biomolecular recognition element (phage). The bound bacteria cause the mass of the resonator to increase, resulting in a decrease in the resonant frequency. For small mass changes ($\Delta m \ll M$), the change in the resonant frequency, Δf, can be approximated by equation 2:

$$\Delta f \approx -\frac{f}{2}\frac{\Delta m}{M} \quad (2)$$

where Δm and M are the mass change and the mass of the sensor, respectively. The frequency is directly proportional to the additional mass attached to the sensor surface, which corresponds to the number of bacterial cells bound to the ME sensor surface. The mass sensitivity (S_m) of the ME sensor is inversely proportional to the initial mass (M) and proportional to the initial resonance frequency (f). It is given as:

$$S_m = \frac{\Delta f}{\Delta m} \approx -\frac{f}{2M} = -\frac{1}{4L^2WT}\sqrt{\frac{E}{\rho^3(1 - v)}} \quad (3)$$

Hence, the mass sensitivity of the ME sensor is largely dependent on the size of the sensor. Decreasing the sensor size improves the sensitivity.

This ME sensor has been successfully applied in both chemical and biological sensing *(7)*. Because the ME sensor is a wireless device without a connection between the sensor and the detection system, multiple sensors can be monitored easily. This study focuses on the rapid, direct detection of *Salmonella* Typhimurium on eggshell surfaces.

MATERIALS AND METHODS

Sensor fabrication and metal deposition. METGLAS 2826MB alloy, obtained from Honeywell International (Morristown, NJ), was used to fabricate the ME resonator platforms for the biosensors. The ribbon was diced into strip-shaped platforms of 1 by 0.2 by 0.028 mm, with an automatic dicing saw (DAD 3220, Disco Corp., Tokyo, Japan). The diamond dicing blade with the thickness of 0.127 mm was used. The dicing speed was 3.00 mm/s, and the spindle speed was 25,000 rpm. Two layers of plastic tape were used to fix the metallic glass samples. The thickness of the tape was 0.105 mm. The platforms were cleaned with acetone and ethanol and annealed at 220°C in vacuum (10^{-3} Torr [~1.3 Pa]) for 2 h. Annealing removes residual stresses generated by the dicing process. Two metal layers (Cr and Au) were then sputter deposited onto all the platform surfaces. The layer of Cr acts as an adhesive interface between the platform and the Au layer. The Au layer provides corrosion resistance and a ready surface for bioprobe immobilization *(5, 11)*.

E2 phage immobilization. The filamentous E2 phage, with highly specific and selective properties toward *Salmonella* Typhimurium, was derived from a landscape f8/8 phage library *(15)*. Dr. James M. Barbaree's laboratory in the Department of Biological Sciences at Auburn University (Auburn, AL) prepared and provided the E2 phage for this research. The E2 phage specific for *Salmonella* Typhimurium was developed by Dr. Valery Petrenko at Auburn University. The concentration of E2 phage solution was 5×10^{11} viruses per ml in a Tris-buffered saline (TBS) solution. TBS is necessary for phage suspension. The resonator platforms were immersed in the phage solution for 1 h. The immobilization of the E2 phage on the platform surface is based on physical adsorption. For the bare gold surface, the filamentous phage coverage is about 50% *(4)*. After the phage immobilization, the biosensors were washed with deionized water twice in order to remove unbound phage and salt originating from the TBS. In order to compensate for the environmental effects and nonspecific binding, control sensors were prepared by following the same steps but without E2 phage immobilization.

BSA blocking. Both measurement (with phage) and control (without phage) sensors were blocked with bovine serum albumin

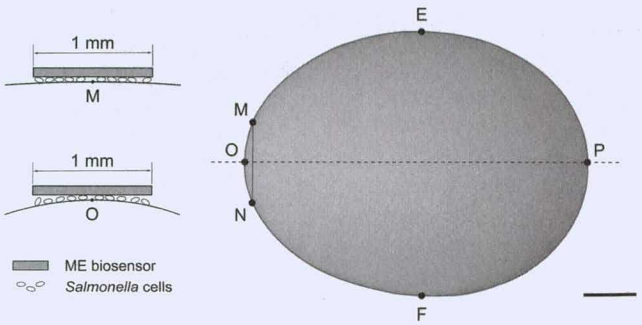

FIGURE 2. *The effect of an egg's surface curvature on the contact area (scale bar: 1 cm). The figures on the left show the contact area around points M and O (not to scale).*

(BSA) to prevent nonspecific binding. The sensors were immersed in a solution of 1 mg/ml BSA for 40 min and washed with deionized water twice.

***Salmonella* detection on eggshell surfaces.** *Salmonella* Typhimurium culture with a concentration of 5×10^8 CFU/ml was provided by Dr. Barbaree's laboratory. The culture solution was stored in a refrigerator at 4°C and equilibrated to room temperature before the test. In this research, the original *Salmonella* Typhimurium solution was diluted to lower concentrations (ranging from 5×10^7 to 5×10^1 CFU/ml) with deionized water. The eggs were purchased from a local supermarket, and the eggshell surfaces were cleaned with deionized water. The *Salmonella* Typhimurium solutions of eight different concentrations (5×10^8 to 5×10^1 CFU/ml) with the same volume (2.5×10^{-2} ml) were inoculated on the shell surface. The contamination area was a 1-cm-diameter circle. This corresponds to the surface *Salmonella* concentration from 1.6×10^7 to 1.6 CFU/cm^2. After the solutions dried for 20 min, both measurement and control sensors were placed on the contaminated shell surface. The eggshells with the sensors were then placed in a humidity-controlled chamber (95%) for 20 min to allow binding of the bacteria to the sensors to occur. Seven measurement sensors and three control sensors were used for each solution concentration. The resonant frequency for each sensor was measured with a network analyzer (model 8751A, HP, Santa Clara, CA, USA) before and after sensors' exposure to the contaminated eggshells. Figure 1 depicts the experimental procedure.

The network analyzer, responsible for the generation of a time-varying external magnetic field and signal detection, was connected to a solenoid coil wound around a glass tube. In order to amplify the frequency signal (output), a static magnetic bias was provided with a bar magnet that was fixed outside of the tube. The test biosensor was placed inside of the coil. The attachment of *Salmonella* Typhimurium cells can be detected by the sensor's resonant frequency change. After the frequency measurements, all sensors and eggshells were exposed to osmium tetroxide (O_SO_4) vapor for 45 min in preparation for scanning electron microscopy (SEM) analysis.

SEM. The osmium-treated sensors were mounted onto an aluminum platform for observation using an SEM (JSM-7000F, JEOL, Tokyo, Japan). SEM observation was performed to confirm *Salmonella* Typhimurium cells were bound to the biosensor surface and, hence, responsible for the observed frequency changes.

RESULTS AND DISCUSSION

The effect of the egg's surface curvature on the contact between the sensors and *Salmonella*. Figure 2

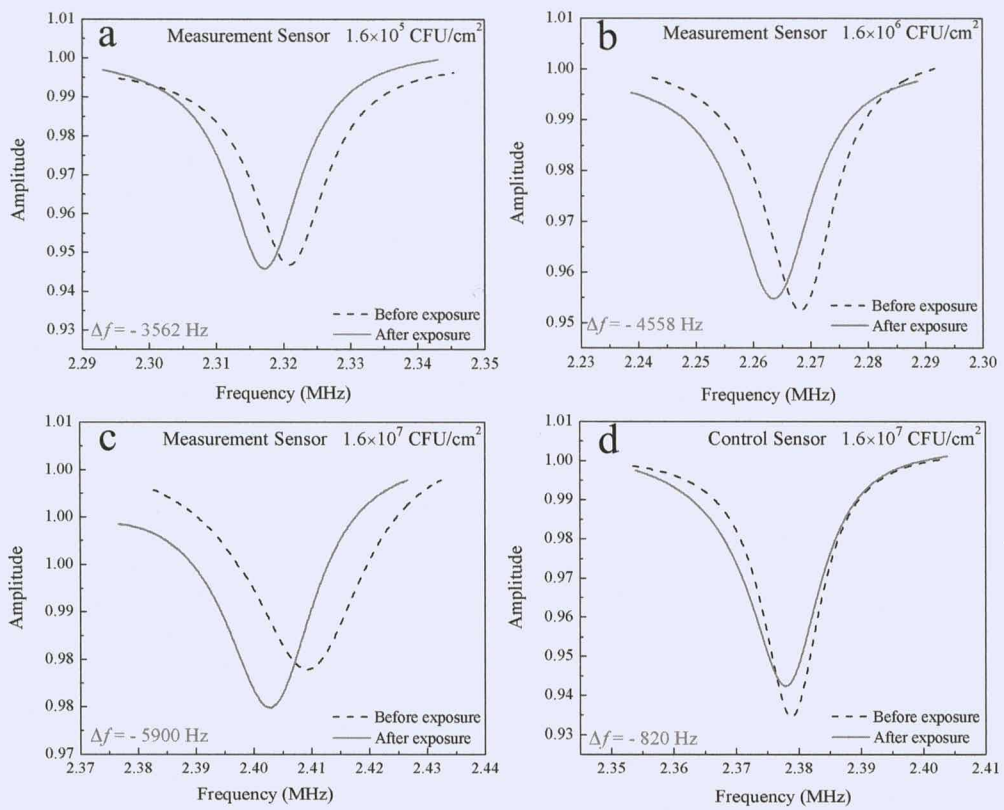

FIGURE 3. *The frequency curves before (dashed line) and after (solid line) exposure to spiked eggshells for ME measurement and control sensors: (a) measurement sensor exposed to 1.6 × 10^7 CFU/cm^2, (b) measurement sensor exposed to 1.6 × 10^6 CFU/cm^2, (c) measurement sensor exposed to 1.6 × 10^5 CFU/cm^2, and (d) control sensor exposed to 1.6 × 10^7 CFU/cm^2.*

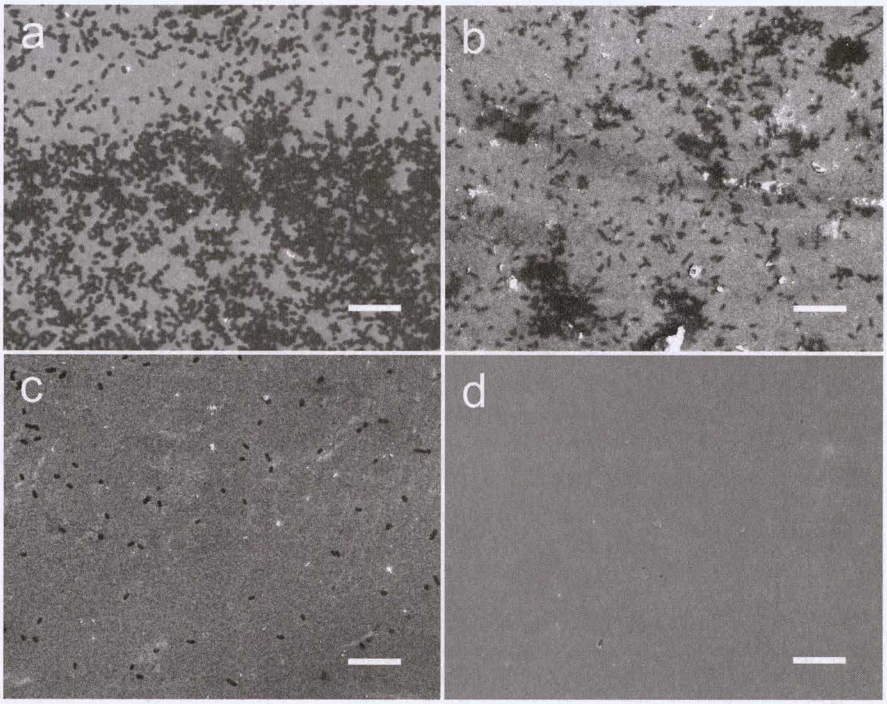

FIGURE 4. *SEM micrographs of sensor surfaces placed on eggshells spiked with different* Salmonella *Typhimurium concentrations: (a) 1.6 × 10^7 CFU/cm^2, (b) 1.6 × 10^6 CFU/cm^2, (c) 1.6 × 10^5 CFU/cm^2, and (d) control sensor exposed to 1.6 × 10^7 CFU/cm^2 (scale bar: 10 μm).*

shows a representative image of the egg's outline, which has nonuniform curvatures. Hence, the surface curvature needs to be evaluated to estimate the contact between a 1-mm-long sensor and *Salmonella* on eggshell. Assuming the egg is axisymmetric around the axis OP, only the top half of the egg outline (i.e., OÊP is considered). The curvature of OÊP decreases from point O, which has the largest curvature, to point E, and increases from point E to point P. In the real situation, the *Salmonella* cells move and assemble with cilia, creating a dynamic layer on the eggshell surface. Based on the curvature calculations with the estimated *Salmonella* layer thickness of larger than 2 μm *(1)*, 100% contact between the sensor and *Salmonella* can be achieved on MÊP, where point M is located between points O and E. The left of Figure 2 shows 100% contact at point M, while less than 100% contact at point O. As the area with larger curvatures (MÔN) is less than 5% of the whole surface, 1-mm-long sensors are able to detect the contamination on over 95% of the eggshell surface.

The detection of *Salmonella* Typhimurium of various concentrations on eggshells. The resonant frequency changes for measurement and control sensors are shown in Figure 3. In the figure, the dashed line demon-

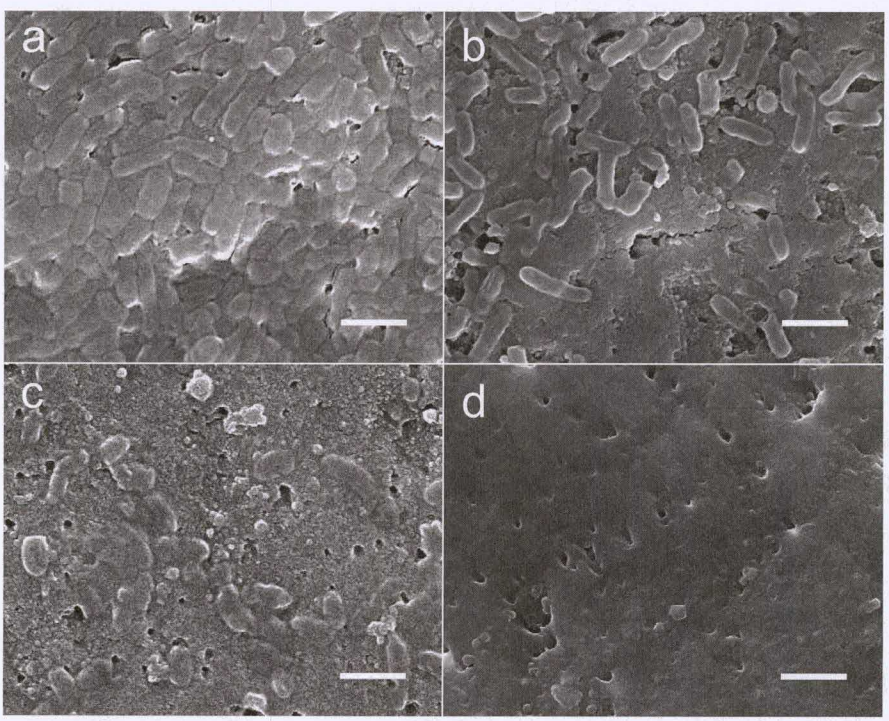

FIGURE 5. *SEM micrographs of eggshell surfaces spiked with different* Salmonella *Typhimurium concentrations: (a) 1.6 × 10^7 CFU/cm^2, (b) 1.6 × 10^6 CFU/cm^2, (c) 1.6 × 10^5 CFU/cm^2, and (d) as-received eggshell, no bacterial spiking (scale bar: 2 μm).*

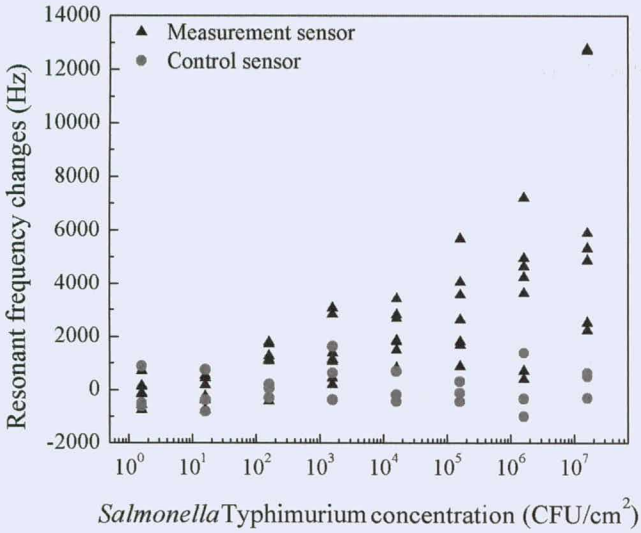

FIGURE 6. *The resonant frequency changes for measurement and control sensors exposed to eggshells spiked with different concentrations of* Salmonella *Typhimurium.*

strates the initial frequency curve of the sensors, whereas the solid line reveals the final frequency curve after the placement of the sensors on *Salmonella*-spiked surfaces. Resonant frequency changes for typical measurement sensors exposed to three different concentrations of *Salmonella* Typhimurium (1.6×10^5 to 1.6×10^7 CFU/cm^2) are shown in Figure 3a through 3c, respectively. This figure displays a decrease of the sensor's initial resonant frequency after binding of *Salmonella* Typhimurium to the sensors. The resonant frequency changes were found to be smaller, as the sensors were exposed to lower concentrations. In other words, the resonant frequency changes were in accordance with the level of *Salmonella* Typhimurium contamination of the eggshells. By contrast, the frequency changes for control sensors were negligible compared with the measurement sensors.

Figure 4 shows representative SEM micrographs for ME biosensors placed on eggshells inoculated with different concentrations of *Salmonella* Typhimurium. The number of *Salmonella* Typhimurium cells on the sensor surfaces decreased as the concentration of the *Salmonella* solutions was decreased. In addition, as shown in Figure 4d, the control sensor surface is much cleaner without bacteria binding. Hence, these SEM micrographs verified that specific binding between the E2 phage and *Salmonella* Typhimurium occurred, resulting in the corresponding large resonant frequency changes of the measurement sensors.

Use of multiple biosensors and the determination of the detection limit. Figure 5 displays SEM micrographs of the eggshell surfaces inoculated with different concentrations of *Salmonella* Typhimurium. Figure 5a through 5c corresponds to the *Salmonella* concentrations of 1.6×10^7 to 1.6×10^5 CFU/cm^2, respectively. Figure 5d is the as-purchased egg surface without *Salmonella* Typhimurium spiking. The SEM microphotographs show the bacterial cells on the surface are not uniformly distributed, especially when the bacterial concentration is low. This is because the cells can migrate and move freely on the humid surface, searching for regions and pores with nutrients and water. Based on this situation, the response of ME biosensors largely depends on where the sensors fall on the eggshell surface. In other words, a single sensor is unlikely to be able to provide contamination information of the whole eggshell when the bacterial cells are nonuniformly distributed on the shell surface. The lower the bacterial concentration is, the more nonuniform the cell distribution is on the surface. Therefore, multiple biosensors need to be used to improve the probability of detection. Multiple biosensors were placed on different areas of the eggshell surface. Figure 6 plots measured frequency changes for both measurement (triangles) and control (circles) biosensors. The resonant frequency changes for measurement biosensors largely depend on the concentration of the *Salmonella* solution. The average frequency changes decreased with decreasing *Salmonella* Typhimurium concentration as anticipated. Furthermore, the resonant frequency changes for

TABLE 1. *Statistical analysis of resonant frequency changes for measurement and control sensors placed on eggshell surfaces*

Salmonella Typhimurium concn (CFU/cm^2)	Type of sensor	Mean (Hz)	SD (Hz)	Probability of Student's *t* test	Confidence level of difference (%)
1.6×10^7	Measurement sensor	6,619.64	4,389.71	0.004176	99.58
	Control sensor	271.00	508.75		
1.6×10^6	Measurement sensor	3,688.90	2,413.65	0.007178	99.28
	Control sensor	12.67	1,225.50		
1.6×10^5	Measurement sensor	2,902.14	1,652.24	0.00127	99.87
	Control sensor	−83.33	376.73		
1.6×10^4	Measurement sensor	2,130.14	886.58	0.002354	99.76
	Control sensor	20.83	590.73		
1.6×10^3	Measurement sensor	1,444.87	1,105.22	0.155241	84.48
	Control sensor	625.00	1,000.00		
1.6×10^2	Measurement sensor	963.43	809.44	0.025457	97.45
	Control sensor	−13.67	255.52		
1.6×10^1	Measurement sensor	−75.96	473.86	0.414938	58.51
	Control sensor	−145.83	806.06		
1.6×10^0	Measurement sensor	−88.21	487.98	0.496555	50.34
	Control sensor	−83.33	832.29		

the control sensors were much smaller and not sensitive to the bacterial solution concentration.

Finally, a statistical analysis based on the one-tail-unpaired Student's *t* test *(13, 16)* was performed to determine the limit of detection. Table 1 summarizes the results of the frequency measurements. As anticipated, there is a variance in the resonant frequency changes of measurement sensors even for the highest spiked concentration. This is because the *Salmonella* Typhimurium on the eggshells were not uniformly distributed and, hence, the measurement depended on where the sensor falls. From the statistical analysis, the detection limit was determined to be 160 CFU/cm^2 for 1-mm-long sensors on the eggshell surfaces.

Unlike previous studies on the detection in bacterial solutions *(3, 6, 7, 9, 18)*, the methodology in this work used direct placement of the sensors on the contaminated food surface and eliminated any preceding sampling procedures facilitated by the wireless nature of detection. In addition, the results demonstrate that the bacterial binding and detection can be realized in a high-humidity environment (95%) within 30 min. In future work, the use of new phages that are specific to different strains of *Salmonella* will be investigated. Food surfaces without cleaning will be tested as real situations.

ACKNOWLEDGMENTS

This work was supported by the Auburn University Detection and Food Safety Center, and was funded by U.S. Department of Agriculture, Cooperative Research and Extension Service grant no. 2005343941567A. The authors greatly appreciate Dr. James M. Barbaree, Dr. Valery Petrenko, and Ms. I.-Hsuan Chen for supplying biological testing solutions.

REFERENCES

1. Arnold, P. 2009. Molecular biology, *Salmonella* bacteria FAQ. Available at: http://www.brighthub.com/science/genetics/articles/50010.aspx. Accessed 24 September 2009.
2. Grimes, C. A., P. G. Stoyanov, D. Kouzoudis, and K. G. Ong. 1999. Remote query pressure measurement using magnetoelastic sensors. *Rev. Sci. Instr.* 70:4711–4714.
3. Guntupalli, R., J. Hu, R. S. Lakshmanan, T. S. Huang, J. M. Barbaree, and B. A. Chin. 2007. A magnetoelastic resonance biosensor immobilized with polyclonal antibody for the detection of *Salmonella* Typhimurium. *Biosens. Bioelectron.* 22:1474–1479.
4. Horikawa, S., D. Bedi, S. Li, W. Shen, S. Huang, I.-H. Chen, Y. Chai, M. L. Auad, M. J. Bozack, J. M. Barbaree, V. A. Petrenko, and B. A. Chin. 2011. Effects of surface functionalization on the surface phage coverage and the subsequent performance of phage-immobilized magnetoelastic biosensors. *Biosens. Bioelectron.* 26:2361–2367.
5. Huang, S., J. Hu, J. Wan, M. L. Johnson, H. Shu, and B. A. Chin. 2007. The effect of annealing and gold deposition on the performance of magnetoelastic biosensors. *Mater. Sci. Eng.* 28:380–386.
6. Huang, S., H. Yang, R. S. Lakshmanan, M. L. Johnson, J. Wan, I.-H. Chen, H. C. Wikle, V. A. Petrenko, J. M. Barbaree, and B. A. Chin. 2009. Sequential detection of *Salmonella* Typhimurium and *Bacillus anthracis* spores using magnetoelastic biosensors. *Biosens. Bioelectron.* 24:1730–1736.
7. Johnson, M. L., J. Wan, S. Huang, Z. Cheng, V. A. Petreko, D. Kim, I.-H. Chen, J. M. Barbaree, J. W. Hong, and B. A. Chin. 2008. A wireless biosensor using microfacbricated phage-interface magnetoelastic particles. *Sens. Actuators A Phys.* 144:38–47.
8. Kim, Y., T. R. Flynn, R. B. Donoff, D. W. Wong, and R. Todd. 2002. Then gene: the polymerase chain reaction and its clinical application. *J. Oral Maxillofac. Surg.* 60:808–815.
9. Lakshmanan, R. S., R. Guntupalli, J. Hu, D. Kim, V. A. Petrenko, J. M. Barbaree, and B. A. Chin. 2007. Phage immobilized magnetoelastic sensor for the detection of *Salmonella* Typhimurium. *J. Microbiol. Methods* 71:55–60.
10. Lequin, R. M. 2005. Enzyme immunoassay (EIA)/enzyme-linked immunosorbent assay (ELISA). *Clin. Chem.* 51:2415–2418.
11. Li, Q., Y. Li, H. Chen, S. Horikawa, W. Shen, A. Simonian, and B. A. Chin. 2010. Direct detection of *Salmonella* Typhimurium on fresh produce using phage-based magnetoelastic biosensors. *Biosens. Bioelectron.* 26:1313–1319.
12. Liang, C., S. Morshed, and B. C. Prorok. 2007. Correction for longitudinal mode vibration in thin slender beams. *Appl. Phys.* 90:22192.
13. Mead, R., R. N. Curnow, and A. M. Hasted. 2003. Statistical methods in agriculture and experimental biology. Chapman and Hall/CRC Press, Boca Raton, FL.
14. Messens, W., L. Duboccage, K. Grijspeerdt, M. Heyndrickx, and L. Herman. 2004. Growth of *Salmonella serovars* in hens' egg albumen as affected by storage prior to inoculation. *Food Microbiol.* 21:25–32.
15. Petrenko, V. A., and I. B. Sorokulova. 2004. Detection of biological threats: a challenge for directed molecular evolution. *J. Microbiol. Methods* 58:147–168.
16. Prescott, D. M. 1977. Methods in cell biology. Academic Press, New York.
17. Schoeni, J. L., K. A. Glass, J. L. McDermott, and A. C. L. Wong. 1995. Growth and penetration of *Salmonella enteritidis, Salmonella heidelberg* and *Salmonella typhimurium* in eggs. *Food Microbiol.* 24: 385–396.
18. Shen, W., R. S. Lakshmanan, L. C. Mathison, V. A. Petrenko, and B. A. Chin. 2009. Phage coated magnetoelastic micro-biosensors for real-time detection of *Bacillus anthracis* spores. *Sens. Actuators B Chem.* 137:501–506.
19. U.S. Food and Drug Administration. 2009. Egg safety final rule. Available at: http://www.fda.gov/Food/FoodSafety/Product-SpecificInformation/EggSafety/EggSafetyActionPlan/ucm170615.htm. Accessed 7 July 2009.
20. U.S. Food and Drug Administration. 2011. Quarterly summary on FDA inspections under the egg safety rule. Available at: http://www.fda.gov/Food/FoodSafety/Product-SpecificInformation/EggSafety/ucm241588.htm. Accessed February 2011.
21. Wilson, S. A., R. P. Jourdain, Q. Zhang, R. A. Dorey, C. R. Bowen, M. Willander, Q. U. Wahab, M. Willander, S. M. Al-hilli, O. Nur, E. Quandt, C. Johansson, E. Pagounis, M. Kohl, J. Matovic, B. Samel, W. Wijngaart, E. W. Jager, D. Carlson, Z. Djinovic, M. Wegener, C. Moldovan, R. Iosub, E. Abad, M. Wendlandt, C. Rusu, and K. Persson. 2007. New materials for micro-scale sensors and actuators an engineering review. *Mater. Sci. Eng. R* 56:1–129.
22. Xiao, X., M. Guo, Q. Li, Q. Cai, S. Yao, and C. A. Grimes. 2008. In situ monitoring of breast cancer cell (MCF-7) growth and quantification of the cytotoxicity of anticancer drugs fluorouracil and cisplatin. *Biosens. Bioelectron.* 24:247–252.
23. Yang, H., and J. Irudayaraj. 2003. Rapid detection of foodborne microorganisms on food surface using Fourier transform Raman spectroscopy. *J. Mol. Struct.* 646:35–43.