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A surface-scanning coil detector for real-time, *in-situ* detection of bacteria on fresh food surfaces

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ABSTRACT

Proof-in-principle of a new surface-scanning coil detector has been demonstrated. This new coil detector excites and measures the resonant frequency of free-standing magnetoelastic (ME) biosensors that may now be placed outside the coil boundaries. With this coil design, the biosensors are no longer required to be placed inside the coil before frequency measurement. Hence, this new coil enables bacterial pathogens to be detected on fresh food surfaces in real-time and *in-situ*. The new coil measurement technique was demonstrated using an E2 phage-coated ME biosensor to detect *Salmonella typhimurium* on tomato surfaces. Real-time, *in-situ* detection was achieved with a limit of detection (LOD) statistically determined to be lower than 1.5×10^3 CFU/mm^2 with a confidence level of difference higher than 95% ($p < 0.05$).

1. Introduction

Every year in the United States over 45 million Americans become ill due to foodborne illness (CDC, 2011). New devices that can be used in the agricultural field, on processing lines, at shipping terminals and in the supermarket/restaurant are needed to detect foodborne pathogens and reduce the suffering and productivity losses resulting from unsafe contaminated food (Jongen, 2005).

Magnetoelastic (ME) biosensors are one device that shows promise for *in-situ*, real-time detection of pathogenic bacteria on food surfaces. The ME biosensor consists of a transducer (ME resonator) that is coated with a bio-molecular recognition element for the specific capture and binding of a pathogenic target bacteria. ME resonators work on the principle of Joule magnetostriction, where the resonator experiences a change in its dimensions in the presence of a magnetic field (Ballantine et al., 1997; Kabos and Stalmachov, 1994). When subjected to a time-varying magnetic field in the direction of the resonator's length, the ME resonator longitudinally vibrates with a characteristic resonant frequency (Li et al., 2012). To form the biosensor, the resonator is coated with a bio-molecular recognition element that is specific to the bacteria being detected. When the ME biosensor comes into contact with the specific target bacteria, the bacteria are captured and bound to the biosensor's surface by the bio-molecular recognition element. This binding, causes an increase in the mass of the sensor that results in a decrease in resonant frequency. This decrease in resonant frequency is proportional to the number of bacterial cells bound to the ME biosensor surface (Li et al., 2012). An electromagnetic coil is used to generate the oscillating magnetic field and measure the biosensor's resonant frequency. Since oscillation and measurement are all controlled through changes in the magnetic field, the ME biosensor is a wireless device and the ME biosensors require no on-board power. A detailed explanation and schematic of the theory of operation can be found in the following references (Kabos and Stalmachov, 1994; Liang et al., 2007; Li et al., 2012).

This paper demonstrates new technology that enables the ME biosensors to be measured at a location outside the boundaries of a solenoid coil. In all research conducted to date, ME biosensors have been placed inside a solenoid coil to measure their resonant frequency (Horikawa et al., 2011; Liang et al., 2007). This limits the use of ME biosensors to small objects or volumes that will fit within the coil. Hence, by enabling measurement outside the coil, *in-situ* measurements on surfaces of any size become possible. In this work, we demonstrate proof-in-principle of a surface-scanning coil detector by measuring bacteria concentration on a food surface using ME biosensors. This technique differs from all previous reports using ME biosensors to measure surface contamination, where retrieval and frequency measurement of the biosensors in the coil were required after exposure to bacteria (Chai et al., 2012; Li et al., 2010; Park et al., 2012).

Fig. 1 compares the differences between the old and new methods of measurement. In both of the methods, a coil is used

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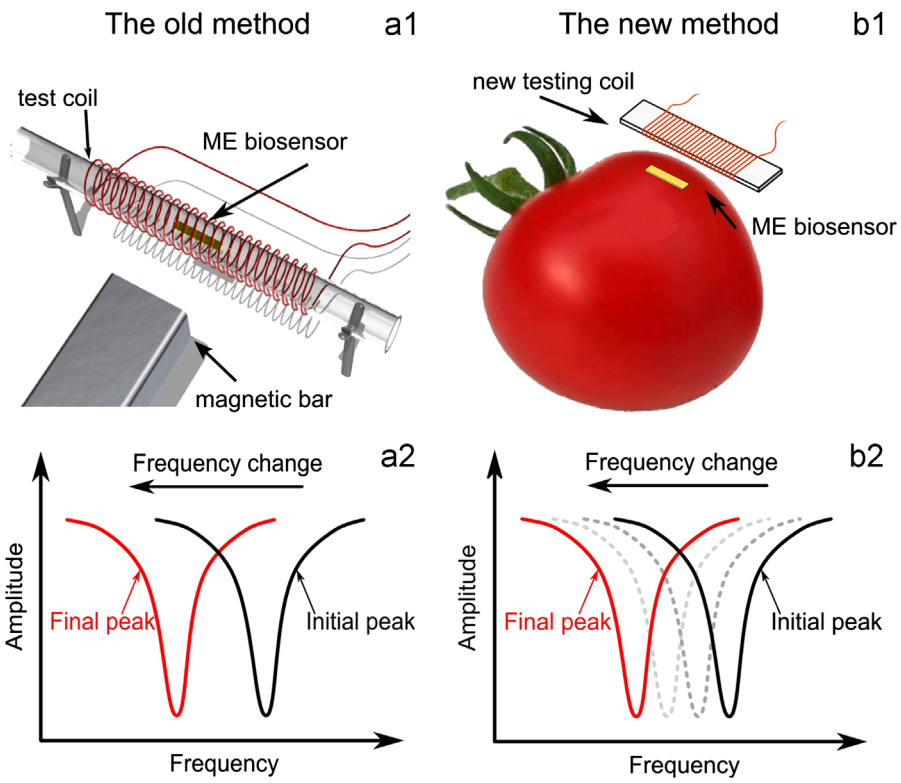

Fig. 1. Comparison between old and new measurement methods: (a1) ME biosensor's old frequency detection method, (a2) the signal of frequency change for the old method, (b1) ME biosensor's detection in new method, (b2) the continuous measurement of frequency change in new experiment.

to create a time-varying magnetic field that excites and detects the biosensor vibration, hence acquiring the biosensor resonant frequency. However, the measurement procedures and signal results are different with the different coil designs. In previous work, the ME biosensors required placement inside the solenoid coil for the frequency measurement (Fig. 1a1), and then they were moved out of the coil for bacteria exposure on the food surface (Chai et al., 2012; Li et al., 2010; Park et al., 2012). Hence, the detection was cumbersome and not real-time because the following three separate steps were required: (1) measurement of the initial resonant frequency of the biosensor inside the coil; (2) exposure to bacteria on the food surface outside of the coil; and (3) placement of the biosensor back inside the coil for the final resonant frequency measurement after bacteria exposure. By contrast, the new detection system shown in Fig. 1b1 enables the measurement of the biosensor frequency directly on a food surface. In this new system, a coil with a rectangular cross-section is utilized to scan the food surface and read the biosensor's response. With the old measurement method, only two frequency measurements were made, before and after bacteria exposure (Fig. 1a2), whereas now continuous, real-time measurements of the resonant frequency can be performed during bacteria exposure (Fig. 1b2).

The ME biosensor has advantages over other microbiological detection techniques, such as enzyme-linked immunosorbent assay (ELISA) (Lequin, 2005) and polymerase chain reaction (PCR) (Miller et al., 2011). First, the ME biosensor method uses phage as the bio-molecular recognition element. Phage has been shown to be highly robust and retain binding affinity even after high temperature storage and use in acid conditions (Petrenko and Vodyanoy, 2003). Food surfaces can be analyzed for multiple target bacteria simultaneously using ME biosensors. Different size ME biosensors may be used with different bio-molecular recognition elements to identify different pathogenic bacteria simultaneously (Huang et al., 2009). ME biosensors are wireless devices and require no on board power. Hence they have been fabricated using standard microelectronic fabrication procedures and are very inexpensive. Over 7,50,000 sensors can be fabricated on a single wafer leading to a cost of less than 1/1000 of a U.S. cent per biosensor (Horikawa, 2013). An in-depth comparison study of the ME biosensor with PCR and the plate culture method for detection of *Salmonella* on tomatoes has been performed. In these experiments the *Salmonella* was grown on the tomatoes to mimic natural environmental conditions. These results showed that the ME biosensor gave similar results to PCR and plate culture method with the ME biosensor being much faster, simpler and less costly than the PCR method (Park et al., 2013a, 2013b). ME biosensors because they can be placed directly on food surfaces for measurement avoid time consuming and often complex water wash procedures (FDA, 2005). Additionally culturing/enrichment steps that are often required are avoided. With further development of the new coil detector design described in this paper, scanning of the surfaces of food *in-situ* for surface bacterial contamination may become a reality.

2. Materials and methods

2.1. Sensor fabrication and metal deposition

METGLAS 2826MB alloy, obtained from Honeywell International, was used to fabricate the ME resonator platforms for the biosensors. The ribbon was diced into strip-shaped platforms of 1 mm × 0.2 mm × 0.028 mm using an automated dicing saw (DAD 3220, Disco Corp, Tokyo, Japan). The platforms were cleaned with acetone and ethanol and annealed at 220 °C in vacuum (10^{-3} Torr) for 2 h. Annealing removes residual stresses generated by the dicing process. Two metal layers (Cr and Au) were then sputter-deposited onto 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 bio-probe immobilization.

2.2. E2 phage immobilization and bovine serum albumin (BSA) surface blocking

Dr. James M. Barbaree's lab in the Department of Biological Sciences at Auburn University prepared and provided the filamentous E2 phage for this research. The E2 phage specific for *S. typhimurium* was developed by Dr. Valery Petrenko at Auburn University. The concentration of E2 phage solution was 5×10^{11} vir/ml in a tris-buffered saline (TBS) solution. TBS was used 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. After the phage immobilization, the biosensors were washed with deionized water twice to remove unbound phage and salt originating from the TBS. To compensate for environmental effects and non-specific binding, control biosensors were prepared following the same steps but without E2 phage immobilization. Both measurement (with phage) and control (without phage) biosensors were blocked with BSA to prevent non-specific binding (Shen et al., 2012). The biosensors were immersed in a 1 mg/ml BSA solution for 40 min and washed with deionized water twice.

2.3. Bacterial contamination on tomato surfaces

Salmonella enterica serovar Typhimurium culture with a concentration of 5×10^8 CFU/ml was provided by Dr. James M. Barbaree's laboratory. The culture solution was stored in a refrigerator at 4 °C and equilibrated to room temperature before use. In this research, the original *S. Typhimurium* solution was serially diluted to lower concentrations (ranging from 5×10^7 CFU/ml to 5×10^1 CFU/ml) with deionized water. Tomatoes were purchased from a local grocery store and the tomato surfaces were cleaned with deionized water. The *S. typhimurium* solutions of six different concentrations (5×10^8 CFU/ml–5×10^3 CFU/ml) with the same volume (2.5×10^{-2} ml) were inoculated onto the tomato surfaces. The contamination area was a circle with a diameter of 3–4 mm. The corresponding surface *Salmonella* concentrations ranged from 1.5×10^6 CFU/mm^2 to 1.5×10^0 CFU/mm^2.

2.4. Real-time, in-situ detection process

Tomatoes were inoculated as described in Section 2.3 above. ME biosensors were then placed on the inoculated regions of the tomato surface and the tomato with biosensors was placed in a humidity-controlled environment (95% RH or 50% RH). The detector coil was then placed above the biosensors during the measurement. Fig. 2a shows the coil structure and bacteria detection on a tomato surface with ME biosensors. The coil was a rectangular cross-section solenoid coil wrapped around a glass core. Based on equivalent circuit calculations and modeling simulations, the coil was designed with 1.3 mm of working length, 1 mm × 0.5 mm of cross-sectional area, and 40 turns of wire. The ME biosensors were located under the coil and on the tomato surface during the entire procedure. An oscillating magnetic field produced outside of the coil actuates longitudinal vibration of the biosensor. The resultant magnetic flux created by the biosensor was then immediately picked up by the same coil, and the resonant frequency was measured (Fig. 2b). The biosensor's resonant frequency was measured at intervals (3, 8, 15, and 25 min) after the biosensors were exposed to *S. typhimurium* on the tomato surface. Fig. 2c and d illustrate the measurement setup. A constant-bias magnetic

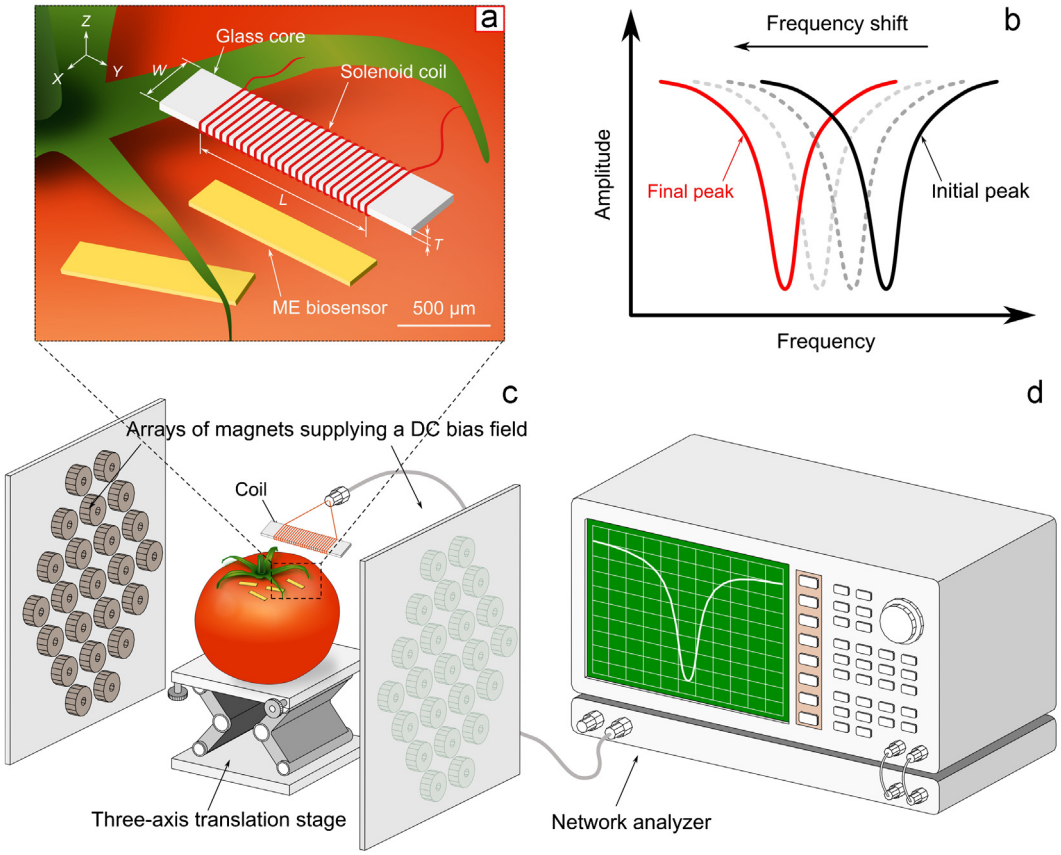

Fig. 2. New methodology for *S. typhimurium* detection on tomato surface.

field was used to amplify the signal. For this purpose, permanent magnets arrayed upon two parallel sheets of ferromagnetic material were set up on two sides of the coil. The magnets created a strong and uniform magnetic field around the biosensor. The detection coil was connected to a network analyzer (Fig. 2d). The network analyzer (HP network analyzer model 8751A) is responsible for generating the oscillating external magnetic field. The input reflection coefficient of the network circuit, S_{11} (S-parameter), was used to measure the signal.

2.5. Scanning electron microscopy

The biosensors were removed from the tomatoes and treated with osmium tetroxide to kill and fix bacteria on their surfaces and then mounted onto an aluminum platform for observation using a scanning electron microscope (SEM) (JSM-7000F, JEOL, Tokyo, Japan). SEM observation was performed to confirm *S. typhimurium* cells were bound to the biosensor surface and, hence, responsible for the observed frequency changes.

3. Results and discussion

3.1. Specificity test of ME biosensor on food surfaces

The filamentous E2 phage, with highly specific and selective properties towards *Salmonella enterica* serovar Typhimurium, was derived from a landscape f8/8 phage library (Lakshmanan et al., 2007; Petrenko and Vodyanoy, 2003; Petrenko et al., 1996). E2 phage has been shown to possess 10- to 1,000-fold greater binding affinity for *S. typhimurium* versus other bacteria (Sorokulova et al. 2005). The specificity of E2 phage-based ME biosensor binding to *S. typhimurium* has also been reported previously (Lakshmanan, 2008; Huang, et al., 2009). Furthermore, Fig. 3 shows the cross-reactivity of E2 phage-based ME biosensors with different bacteria on food surfaces (*S. typhimurium*, *Bacillus anthracis* spores, *Eschericia coli* and *Listeria monocytogenes*). In addition, ME biosensors were also tested on bare surface as a reference. There are ten samples for each group and the standard deviation is shown as the error bar. Hence, the results of resonant frequency changes in Fig. 3 shows E2 phage-based ME biosensors have highly specific binding with *S. typhimurium*.

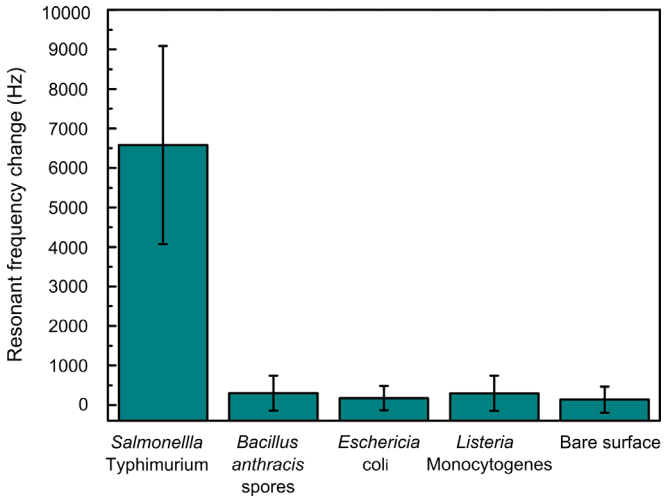

Fig. 3. Cross-reactivity of E2 phage-based ME biosensors with different bacteria (The reference is a bare tomato surface without bacteria).

3.2. Real-time detection and humidity effects

The real-time response of ME biosensors exposed to *S. typhimurium* on tomato surfaces was examined. Measurement and control biosensors were placed upon a tomato surface for bacteria exposure, and the biosensors' resonant frequency was measured with time. Fig. 4 represents the real-time frequency changes during exposure to *S. typhimurium* at a concentration of 1.5×10^6 CFU/mm^2 in 95% and 50% RH environments. In Fig. 4, the solid line shows the frequency changes of one sample of measurement biosensor during bacteria exposure, while the dashed line shows a control biosensor. Error bars which represent the measurement error from the environment and resolution of the detection system are shown on each test point. Fig. 4a shows the biosensor's frequency changes in a high humidity condition (95% RH). The result showed the frequency from 0 to 10 min had a large change. After 15 min, the frequency reached the upper plateau and remained relatively constant. This result indicates the binding reaction between the E2 phage and *Salmonella* cells is not an instantaneous process; and the detection time should be about 15 min for the ME biosensors.

The environmental humidity has a significant effect on the reaction between E2 phage and *S. typhimurium*. Fig. 4a and b are

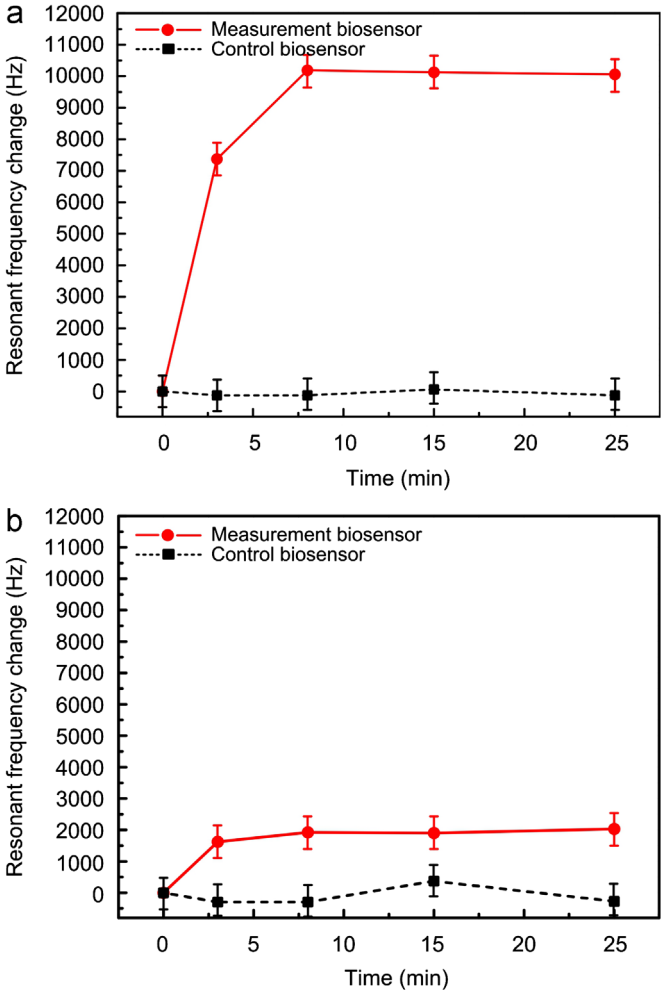

Fig. 4. Frequency changes with time at different humidites: (a) 95% RH and (b) 50% RH (Solid curves show one sample of the measurement biosensor response, while dashed curves show one sample of the control biosensor response).

resonant frequency measurements in humid environments of 95% RH and 50% RH, respectively. The control biosensors for both conditions have similar frequency changes. However, the measurement biosensor in 95% RH environment show much greater

frequency changes than that tested in the 50% RH environment. For the measurement biosensors, the resonant frequency change is approximately 10,000 Hz at 95% RH and 2,000 Hz at 50% RH. Hence, the higher humidity led to greater binding between the phage and bacterial cells.

There are two reasons for the effect of humidity on the specific binding reaction. First, water is the medium for the movement of the bacteria cells (Sorokulova et al., 2005). With less water available, the phage and bacteria have a lower opportunity to encounter and bind. Second, a high humidity environment helps maintain the three-dimensional structure of the functional protein in the phage (Kast and Hilvert, 1997). Specifically, the pVIII protein, which is the functional protein of E2 phage for the specific binding with *S. typhimurium*, may lose the binding affinity with an altered structure in low humidity (Kuzmicheva et al., 2009).

3.3. Food contamination detection with ME biosensors in different *S. typhimurium* concentrations

The effect of *S. typhimurium* concentration on the biosensor response was studied. Three different bacteria surface concentrations (1.5×10^2, 1.5×10^4 and 1.5×10^6 CFU/mm^2) were inoculated on tomato surfaces, and the biosensors' resonant frequencies measured. In this experiment, the relative humidity was maintained at 95%. Fig. 5 displays the frequency changes of the measurement and control biosensors with time for the different bacteria concentrations. As can be seen, the frequency change settled to the final value within 15 min of exposure, with greater frequency changes as bacteria concentration increased. The SEM micrographs (on the right in Fig. 5) show the surface of the measurement biosensors with the captured bacteria cells. These

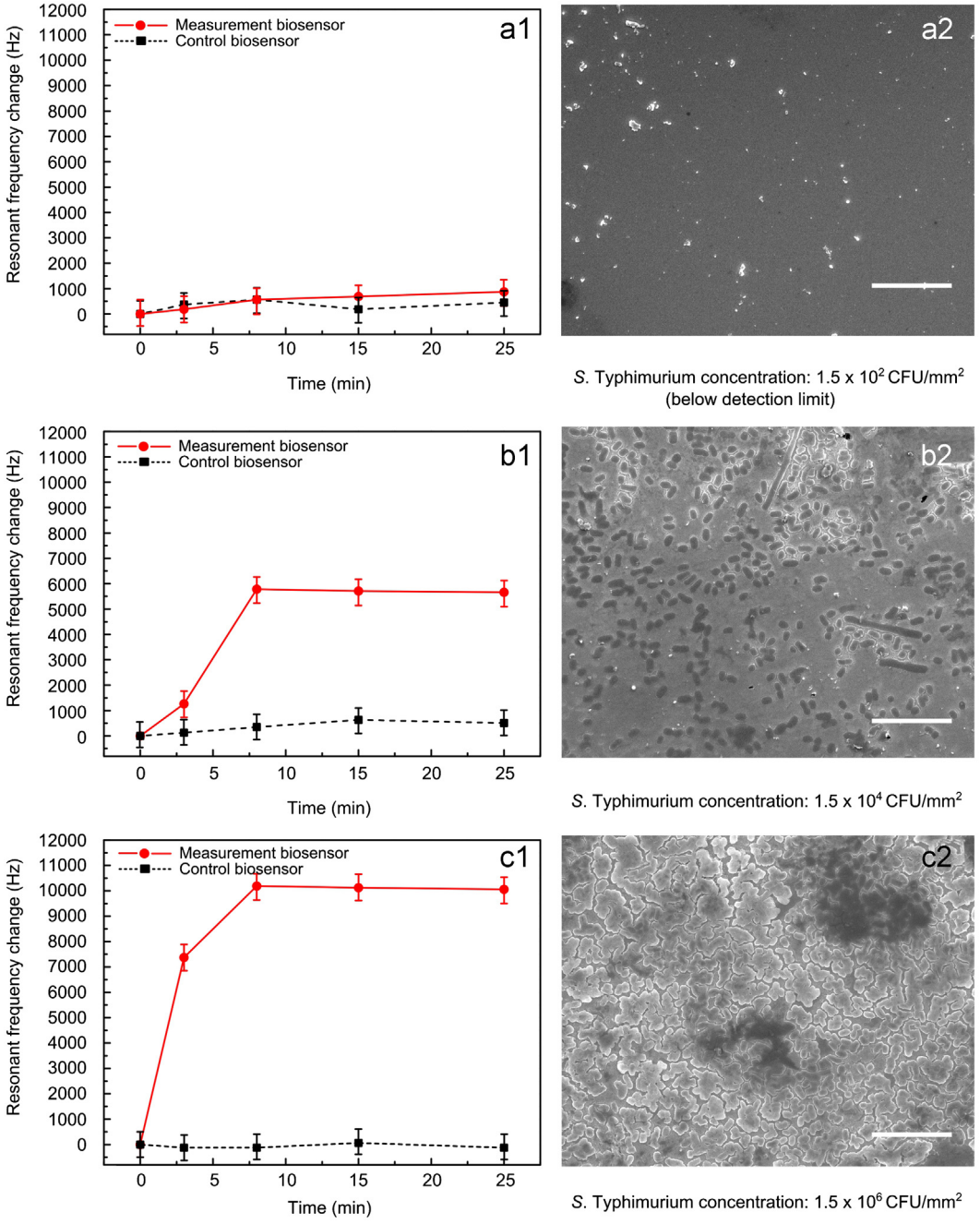

Fig. 5. Resonant frequency changes with time for different *S. Typhimurium* concentrations: (a) 1.5×10^2 CFU/mm^2, (b) 1.5×10^4 CFU/mm^2 and (c) 1.5×10^6 CFU/mm^2 (Measurement bar: 10 μm;environmental humidity: 95% RH).

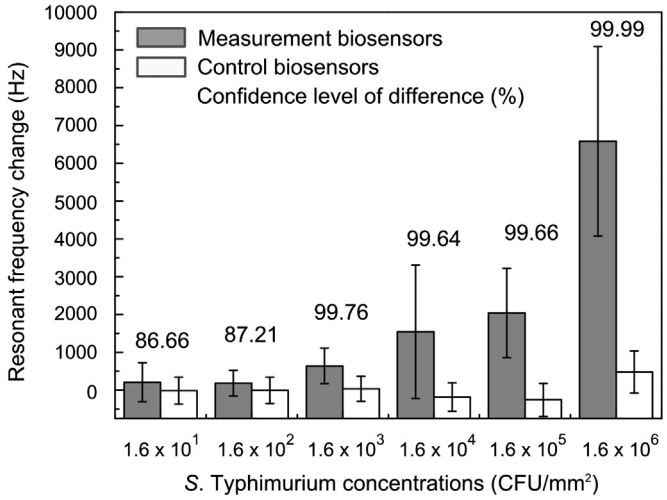

Fig. 6. Resonant frequency changes for measurement and control biosensors placed on tomato surfaces inoculated with different concentrations of *S. typhimurium*.

micrographs corroborate the frequency change results and demonstrate that the ME biosensors captured more bacteria cells as the bacteria concentrations increased. For the control biosensors, the frequency changes do not show obvious differences for different concentrations. This demonstrates that specific binding to the bacteria occurred on the measurement biosensors. In short, this methodology demonstrates the specific and quantitative real-time detection on fresh food surfaces.

Previous research has shown that the bacterial cells on food surfaces are not uniformly distributed (Li et al., 2010). This is because the cells can migrate and move freely on moist surfaces. Hence, the response of the ME biosensors largely depends on where the biosensors fall on the tomato surface. Therefore, a single biosensor is unlikely to be able to provide contamination information for the whole tomato. Multiple biosensors, thus, need to be employed to improve the probability of detection. The probability of detection is dependent on several factors, including the number of biosensors, biosensor size, and the roughness of food surfaces (Horikawa, 2013).

In order to study the detection limit of the new detection system, the *in-situ* biosensor response of ten measurement biosensors and ten control biosensors were randomly placed on the contaminated surfaces and measured at each bacteria surface concentration (from 1.5×10^1 to 1.5×10^6 CFU/mm^2). A plot of the frequency changes for both the measurement (solid circles) and control (open triangles) biosensors in different bacteria concentrations is shown in Fig. 6. The data points are the arithmetic mean value with error bars representing the standard deviation. In addition, a Student's *t*-test calculation was performed to analyze the degree of dissimilarity between the measurement and control biosensors and is shown at each concentration. Fig. 6 illustrates that the resonant frequency changes of the measurement biosensors were found to be largely dependent on the surface density of *S. typhimurium* (Mead et al., 2003). By contrast, the control biosensors showed much smaller responses, indicating that selective binding of *S. typhimurium* on the measurement biosensors occurred. Meanwhile, the variance in the resonant frequency changes of the measurement biosensors is due to the non-uniform distribution of *S. typhimurium* on the tomato surface (Li et al., 2010). Increasing the number of biosensors and decreasing the biosensor's size can improve the repeatability of the detection on food surfaces (Horikawa, 2013). From the *t*-test analysis, these responses of the measurement and control biosensors were found to be different down to 1.5×10^3 CFU/mm^2 with a confidence level of difference higher than 95% ($p < 0.05$).

4. Conclusions

Proof-in-principle of a new coil design was demonstrated. This new coil was used to excite and measure the ME biosensor outside the boundaries of the detection coil, enabling continuous measurements of ME biosensors placed upon surfaces. A gradual change of the resonant frequency was observed over time during the reaction between an E2 phage-coated ME biosensor and *S. typhimurium* on a tomato surface. Real-time, *in-situ* detection was thus achieved with a ME biosensor. The research shows that the bacteria reaction and biosensor detection process depends on environmental humidity. The LOD was statistically determined to be lower than 1.5×10^3 CFU/mm^2 with a confidence level of difference higher than 95% ($p < 0.05$). The LOD can be improved by increasing the number of biosensors and decreasing the biosensor's size (Horikawa, 2013).

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2013.06.056.

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