Biosensors and Bioelectronics 50 (2013) 311–317

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

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

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ARTICLE INFO

Article history:
Received 31 March 2013
Received in revised form
10 June 2013
Accepted 26 June 2013
Available online 9 July 2013

Keywords:
Surface-scanning coil detector
Magnetoelastic biosensor
Real-time detection
In-situ detection
Salmonella typhimurium
Fresh food surfaces

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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http://dx.doi.org/10.1016/j.bios.2013.06.056

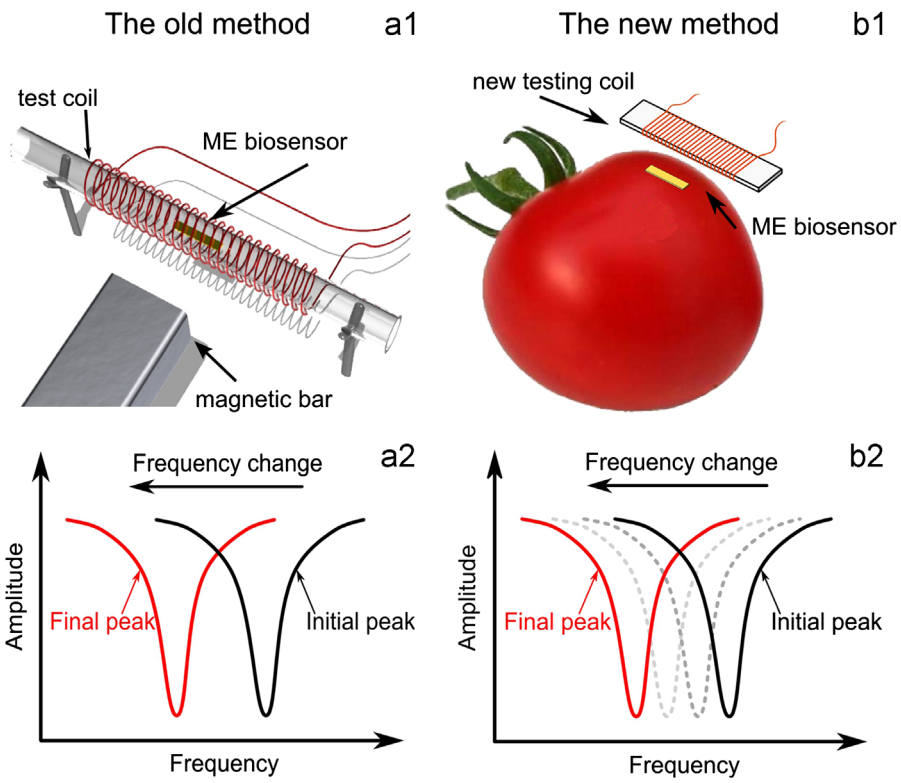

Fig. 1. Comparison between old and new measurement methods: (a1) ME biosensor's old frequency detection method, (a2) the signal of frequency change for the old method, (b1) ME biosensor's detection in new method, (b2) the continuous measurement of frequency change in new experiment.

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