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Comparative study of thermal stability of magnetostrictive biosensor between two kinds of biorecognition elements

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ABSTRACT

Magnetostrictive biosensors specific to *Salmonella typhimurium* were prepared by immobilizing antibody or phage as biorecognition elements onto the magnetostrictive sensor platform. The sensors were stored at temperatures of 25 °C (room temperature), 45 °C and 65 °C, respectively, and the ability to bind *S. typhimurium* was detected by testing the resonant frequency shift using a HP network analyzer after exposure to 1 mL of 1×10^9 cfu/mL of *S. typhimurium* at a predetermined schedule. The binding of *S. typhimurium* to biosensors was confirmed by Scanning Electron Microscopy (SEM). The results showed that there existed an initial sudden drop in the average density of *S. typhimurium* bound to the biosensor surface versus duration at different temperatures for the two kinds of recognition elements, and the binding ability to *S. typhimurium* of phage-immobilized biosensors was much better than that of antibody-immobilized biosensors, with longevity longer than 30 days at all tested temperatures, though decreasing gradually over the testing period. While the longevity of antibody-immobilized biosensors was only about 30, 8 and 5 days at room temperature (25 °C), 45 °C and 65 °C, respectively. Meanwhile, the activation energy of the two kinds of biosensors was investigated, and it was found that phage immobilized sensors showed much higher activation energy than antibody immobilized sensors, which resulted in less dependency on temperature and thus having much better thermal stability than antibody immobilized sensors.

1. Introduction

Salmonella typhimurium, a kind of food-borne pathogen, is one of the greatest concerns related to fresh fruits and vegetables [1]. The symptoms of *Salmonella* infection are diarrhea (88%), fever (80%), abdominal cramps (65%), nausea (42%), occasional vomiting (35%), and headache (29%) [2]. The contamination of fresh food with *Salmonella* can occur at any point from the farm to the table, and the amount of consumption of poultry products, milk and vegetables is getting more and more for recent years, therefore it would be desirable and urgent for fresh food to be systematically detected using biosensor during production and distribution [3–7].

A biosensor essentially consists of two main components viz., a physical transducer and a biorecognition element. In this study, a magnetostrictive platform served as the transducer, since it offers wireless or remote detecting, which is a unique advantage over conventional sensor platforms, and polyclonal antibody or filamentous phage was used as the biorecognition element.

Antibodies are immune system-related proteins called immunoglobulins, which can selectively bind target antigen because of their special structure and different amino acid sequence. However antibodies are generally fragile and filamentous phage has emerged recently as a substitute to antibodies [8–11] as bio-recognition elements for biosensors owing to their environmental robustness [12].

For all practical applications, it is very essential for both major components to be robust enough to withstand the rigors of the field conditions. However, in most cases, the biorecognition element is quite susceptible to suffer from the changes of the field environment. Hence it is of utmost importance to test the stability of the bio-recognition element. Though some literatures found about the thermal stability of free biorecognition element [3,13,14], no report found about those immobilized on a sensor platform. Since the immobilization may alter their stability, it is necessary to investigate the stability of the two kinds of bio-recognition elements at three different temperatures after immobilizing on a magnetostrictive sensor platform, and make a comparison analysis of them.

2. Materials and methods

2.1. Materials and cultures

METGLAS® 2826 MB alloy (Conway, SC) in the form of a long roll of ribbon obtained from Honeywell International was used as the sensor platform; the composition is $Fe_{40}Ni_{38}Mo_4B_{18}$. Magnetostrictive sensors

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with the size of 5 mm × 1 mm were cut using an auto-controlled micro dicing saw. The diced sensors were ultrasonically cleaned in methanol solution to remove grease and debris left by the dicing process followed by a thermal anneal in a vacuum oven at 200 °C for 2 h. To improve the environmental stability as well as the bioactivity of the biosensors, thin layers of chromium and gold were coated on both sides of the sensors using a Denton™ (Moorestown, NJ) high vacuum RF sputtering system.

Filamentous phage clone E2 used in this research was derived from a landscape f8/8 phage library [10], which has been studied and verified to be highly specific and selective towards *S. typhimurium* [15]. Polyclonal antibody to *S. typhimurium* which was immune system-related proteins called immunoglobulins was purchased from Abcam Inc (Cambridge, MA) with the concentration of 1 mg/mL, which exhibited good specificity to *Salmonella* bacteria [16]. Subsequent dilution of the suspensions was performed using TBS solution. *S. typhimurium* cultures (1×10^9 cfu/mL) were prepared in the Department of Life Science at Auburn University. All test solutions were maintained at 4 °C before using.

2.2. Principle of detection

Due to the magnetoelastic nature of the amorphous magnetostrictive alloy, the sensor exhibits a physical resonance when it undergoes a time-varying magnetic field, thus emitting magnetic flux, and this can be monitored remotely without the use of direct physical connections. As Fig. 1 denotes, the frequency spectrum of the sensor can be obtained by sweeping an AC magnetic interrogation field over a predetermined frequency range while monitoring the response of the sensor using a pickup coil. If the frequency of the AC field is equal to the mechanical resonant frequency of the sensor, the conversion of the magnetic energy into elastic energy is maximal and the sensor undergoes a magnetostrictive resonance. For a thin, ribbon-shaped sensor of length L vibrating in its basal plane the fundamental resonant frequency of the longitudinal vibrations is described by [17],

$$f = \sqrt{E/\rho \cdot (1-\sigma^2)}/2L \tag{1}$$

where E denotes Young's modulus of elasticity, σ is the Poisson's ratio, ρ is the density of the sensor material, and L is the long dimension of the sensor. Due to the shape demagnetizing factors of the ribbon-like sensor the magnetic permeability is greatest along its length; hence an incident magnetic field will generate longitudinal vibrations in the sensor from almost any orientation except normal to the basal plane of the sensor.

When the testing temperature, humidity and other environmental parameters are constant, the resonant frequency change of the magnetostrictive sensor depends only on the mass change on its surface. An additional mass Δm to the mass of the sensor M corresponds to a change in the resonant frequency. If the mass increase is small compared to the mass of the sensor the shift in the resonant frequency is given by [18],

$$\Delta f = -f \cdot \Delta m/2 \cdot M \tag{2}$$

where f is the initial resonant frequency, M is the initial mass, Δm is the mass change, and Δf is the shift in the resonant frequency of the sensor.

Eq. (2) shows that the resonant frequency shifts linearly and decreases with increasing mass on the sensor surface. Therefore capturing of the target organism onto the surface of the biosensor causes a mass increase with a corresponding decrease in its resonant frequency. Magnetostrictive materials have been used to develop environmental as well as chemical sensors [19–23]. This study has shown that, by employing a suitable bioprobe for detecting pathogens, this material can be used as a biosensor.

2.3. Immobilization of biorecognition elements

The Langmuir–Blodgett (LB) technique was used for antibody immobilization on the magnetostrictive sensors. Seven monolayers containing antibodies are transferred onto the magnetostrictive sensor surface using a LB film balance KSV 2200 LB (KSV Chemicals, Finland). Phage was immobilized on sensors using physical adsorption by immersing the sensor platform in a phage suspension for 1 h. The ME resonator platforms were then washed with 1× TBS buffer and sterilized distilled water (DW) in order to remove any unbound bioprobe and debris. After immobilization, any unbound area of the ME resonator platform was blocked with 300 μL of 1% bovine serum albumin (BSA, Sigma-Aldrich Co., St. Louis, MO, USA) at 22 °C for 1 h. Finally, the ME resonator platform was washed three times with sterilized DW and then dried in air.

2.4. Thermal stability experimental

After immobilization, the sensors were divided into 3 sets and maintained in 3 constant temperature humidity chambers with temperatures of 25 °C, 45 °C and 65 °C, respectively. At the predetermined schedule, six sensors from each of the three sets were taken to undergo testing and sensors were allowed to attain room temperature before testing. They were immersed in *S. typhimurium* water solution with the volume of 1 mL and the concentration of 1×10^9 cfu/mL for 30 min to bind bacterial cells. The resonant frequency of the sensors was measured using a HP network analyzer 8751A with S-parameter test set at 87511A before and after binding of bacterial cells to the surface of the sensor. Then the sensors were removed and exposed to Osmium tetraoxide (OsO_4) vapor for 1 h to fix the bacterial cell wall to facilitate Scanning Electron Microscopy (SEM) observations. Finally, SEM images were examined

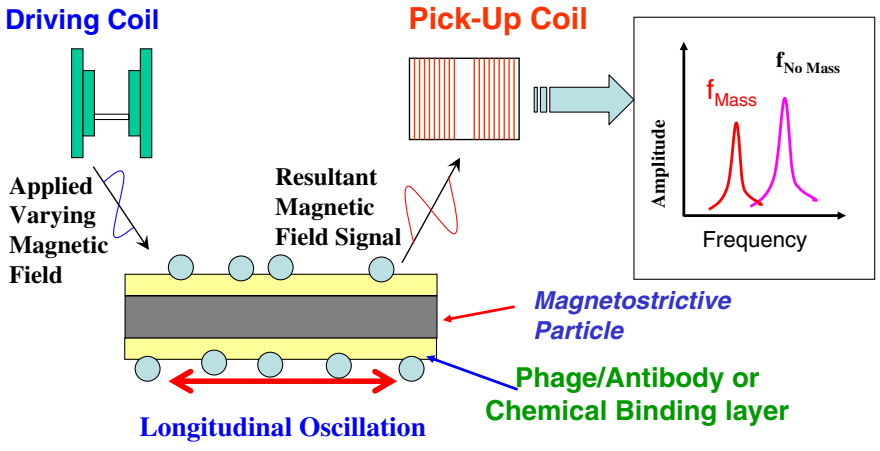

Fig. 1. Schematic illustration of the wireless nature of the magnetostrictive biosensors and its basic principle for bacteria detection.