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Amorphous magnetoelastic sensors for the detection of biological agents

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ABSTRACT

Freestanding amorphous magnetoelastic (ME) biosensors were fabricated by two ways. One type with larger size, $2000 \times 400 \times 15$ μm, $1000 \times 200 \times 15$ μm and $500 \times 100 \times 15$ μm, was made from an ME Fe40Ni38Mo4B18 ribbon, the other with smaller size $200 \times 40 \times 4$ μm was manufactured by dual beam sputtering and non-traditional microelectronic fabrication techniques. Both platforms were immobilized with JRB7 phage and were developed for the real-time in vitro detection of *Bacillus anthracis* spores. The experimental results show that the measured sensitivity of the ME sensors agrees with theoretical predictions and the specificity of ME sensors coated with JRB7 phage for *B. anthracis* spore species is excellent. The $200 \times 40 \times 4$ μm biosensor was found to have a detection limit of 10^2 cfu/ml and sensitivity of 13.1 kHz/decade.

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1. Introduction

In 2001, US Citizens were terrorized by deliberate exposure to *Bacillus anthracis* spores that are the causative agent for anthrax during terrorist attacks targeting the US Postal Service. This attack led to the exposure of 16 people and death of 5. Mass panic amongst the general public led to the realization that bioterrorism could greatly disrupt both the commercial and agricultural sectors of the US economy. Thus it is critical for national security to detect early those biological warfare agents in very small amounts or very low concentrations.

When an alternating magnetic field is applied to an amorphous magnetoelastic (ME) material, it undergoes a corresponding oscillating shape change that gives rise to a mechanical vibration with a characteristic resonance frequency. This frequency is a function of the shape, physical dimensions, and mass of the sensor platform. If *B. anthracis* spores bind to the surface of a platform made of ME material (attachment of mass), a drop in the platform resonance frequency will be induced. Changes in the frequency can be measured wirelessly and remotely both in air and in liquid. Based upon this, freestanding ME sensors for the detection of *B. anthracis* spores can be developed. These sensors are not only accurate, fast, and inexpensive but are also more sensitive and can be used in the field, unlike traditional identification methods, including plate culture, polymerase chain reaction, and enzyme-linked immunosorbent assays [1–5]. Binding of the target analyte can be achieved by immobilizing a selective and specific functional layer on the outer surface of the platform for sensor. The principle for this type of sensor is shown in Fig. 1.

In this article we report on the fabrication and characterization of freestanding wireless amorphous magnetoelastic sensors for the detection of *B. anthracis* spores that are the causative agent for anthrax.

2. Experimental

2.1. Sensor fabrication

2.1.1. Sensors made from ME ribbon

Commercially available ME materials (Metglas alloy 2826 MB) in ribbon form from Metglas, Inc. (Conway, SC) were employed to fabricate one type of ME sensor. The average composition of the ribbon was Fe40Ni38Mo4B18. A small piece was cut from the ribbons and hand polished to a thickness of 15 μm by standard metallographic preparation techniques. The ME material pieces were then washed ultrasonically with ethanol, and then diced into rectangular particles of three different sizes, 2000×400 μm, 1000×200 μm and 500×100 μm, using a micro-dicing saw. The particles were then annealed at 200 °C for 2 h in a vacuum furnace to relieve residual stress. After annealing these pieces were coated

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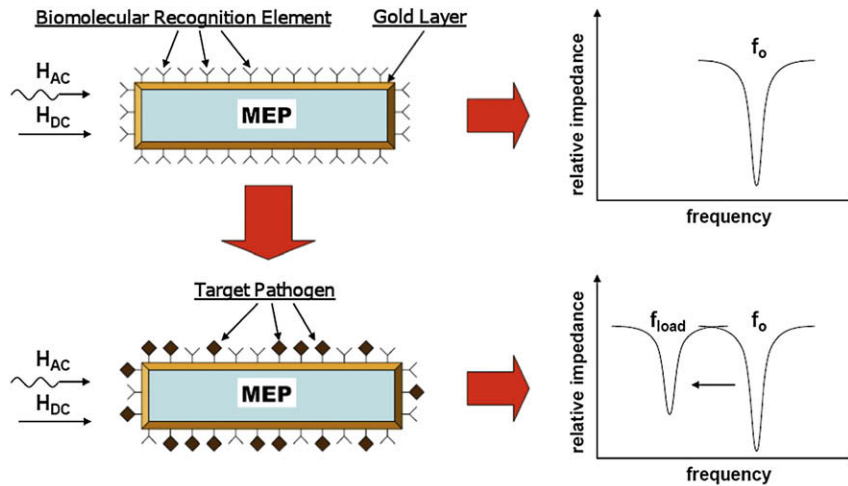

Fig. 1. Diagram of the operating principle of a magnetoelastic particle (MEP) biosensor.

with chromium and gold thin films by a magnetron sputtering system. The Cr thin film works as an adhesive interlayer between the gold and the ME material. The gold surface improves the sensor resistance to corrosion and provides a ready surface for bio-probe immobilization.

2.1.2. *Sensors made from ME films*

In order to achieve detection of low spore/cell counts, ME particles with smaller size, $200 \times 40 \times 4$ μm, were fabricated using microelectronic processes including a co-sputtering and a non-traditional lift-off process. Amorphous-thick films with compositions near to 80/20 at.% iron/boron were magnetron sputtered by a Discovery-18 sputter system from Denton Vacuum USA (Moorestown, NJ) onto silicon wafers at a base pressure 7×10^{-7} Torr. The fabrication process starts with a plain silicon wafer that was sputter coated with a layer of Cr and Au, each at a thickness of 30–40 nm. Then the wafer was patterned with rectangular particles of 200×40 μm using photolithography processes. A thin gold film was then deposited onto the substrate, which works as a bottom protective layer for the particles. Fe (DC) and B (RF) targets, on separate cathodes, were used simultaneously to deposit the iron–boron alloy onto the wafer. A DC power of 42 W and a RF power of 101 W were used for depositing amorphous-thick films with compositions around 80/20 at.% iron/boron. An average film thickness of 4 μm was achieved with a deposition rate of about 4.2 nm/min. A final gold thin film was coated on the substrate as the top protective layer for the particles. This layer also functions as a ready surface for biological immobilization. A lift-off process employing a wash with solvent was used to remove the particles from the wafer. These particles were cleaned with acetone. The as-sputtered particles were annealed in a vacuum oven at 200 °C for about 3 h. This temperature was chosen because it is high enough such that the as-sputtered defects may be healed within a reasonable time, yet it is still far below the Curie temperature of 374 °C [6], as well as the recrystallization temperature, which may vary between 390 and 460 °C, depending on exact boron content [7,8].

2.2. *Phage immobilization*

The bio-recognition element used was a filamentous phage selected specifically for *B. anthracis* spores, clone JRB7. JRB7 was developed by the Department of Pathobiology at Auburn University [9]. This JRB7 phage was immobilized onto the gold surface of the measurement sensor by immersing the gold-coated ME particles in the JRB7 phage solution (10^{11} vir/ml) for 1 h. Then the sensors were

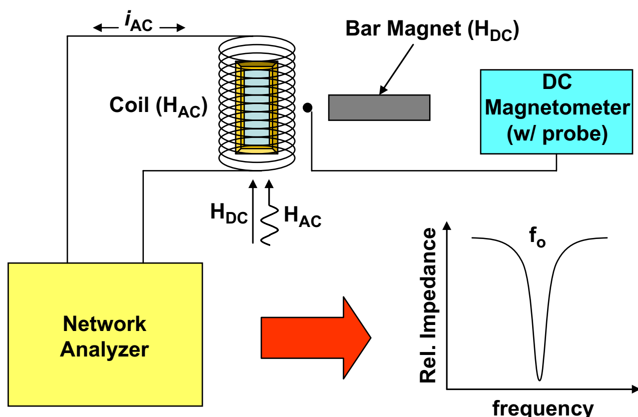

Fig. 2. Diagram of the characterization method for the determination of magnetostrictive particle resonance frequency.

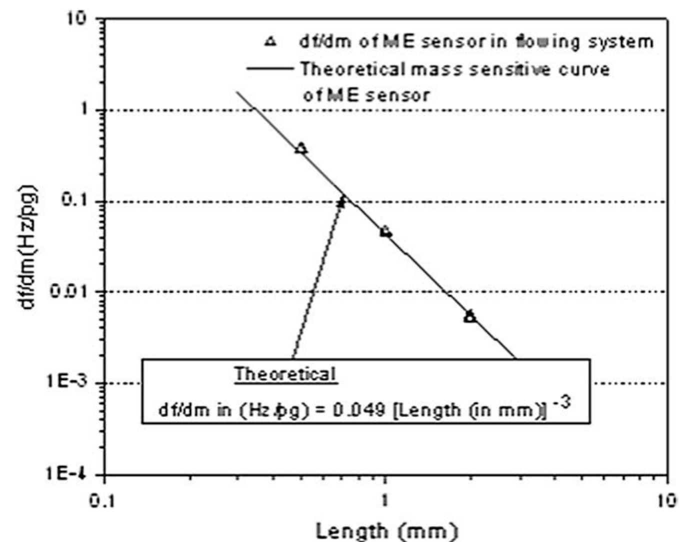

Fig. 3. Mass sensitivity of ME sensor for a flowing analyte.

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