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Phage coated magnetoelastic micro-biosensors for real-time detection of *Bacillus anthracis* spores☆

Wen Shen[a,*], Ramji S. Lakshmanan[a], Leslie C. Mathison[a], Valery A. Petrenko[b], Bryan A. Chin[a]

[a] *Materials Research & Education Center, Auburn University, 275 Wilmore Labs, Auburn, AL 36849, USA*
[b] *Department of Pathobiology, Auburn University, Auburn, AL 36849, USA*

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ABSTRACT

A micro-scale, freestanding, magnetoelastic biosensor coated with phage has been developed for the real-time in vitro detection of *Bacillus anthracis* spores. The sensor exhibits a characteristic resonance frequency upon the application of an alternating external magnetic field. It has a high sensitivity to the change in mass when spores are attached. The frequency versus mass sensitivity increases significantly with a decrease in sensor length. Spore detection is realized by measuring the resonance frequency change due to the change in mass as spores are captured onto the sensor surface. *B. anthracis* spore suspensions in a range of concentration levels (5×10^1 to 5×10^8 spores/ml) was tested using a 1000 μm × 200 μm × 15 μm sensor in a flowing fluid at a flow rate of 40 μl/min. The binding kinetics was analyzed based on the attachment rate. The specificity of the sensor to *B. anthracis* spores was examined compared with other *Bacillus* species.

1. Introduction

Bacillus anthracis spores have become well-known biological warfare agents because of their ability to cause mortality in humans and their long life under unfavorable environments [1]. To guard against this threat, it is necessary to have a method of detection that is very rapid, very sensitive, and small enough that it can be taken to the site of possible contamination and give results without requiring extensive training of operating personnel.

The conventional methods of detection use antibodies and peptides as bio-molecular recognition elements. These methods tend to be very expensive, take a long time to obtain results, are not very sensitive or may not be selective [2–4]. An alternate and newer method has been developed at Auburn University for detecting the *B. anthracis* spores [5–7]. This new method uses phages (clone JRB7 from a phage library developed at Auburn University in Alabama) that are very specific for binding with *B. anthracis* spores. Compared with antibodies, these phages serve as excellent binding agents due to their low cost, simple operation, and high specificity to the target spore or bacterium type while rejecting other strains of anthracis or non-target bacterium. By using a phage developed to specifically capture *B. anthracis* spores, detection can be done in minutes using machines that can be transported to the site of interest [8,9].

Very often, bacteria and spores exist in liquids. To reliably detect low concentrations, it is necessary to bring the bacteria to the sensor. The method proposed here uses a flowing liquid to achieve a more rapid and reliable response than could be achieved by using sensors in static liquid. Previous research using static liquid was used to verify the change in frequency versus mass of very small sensors using phage [9].

Unlike traditional acoustic wave devices [10] that require complex wiring for power and measurement of the transducer, the sensor proposed here is wireless, free standing, robust in both air and liquid and has a rapid response which allows real-time detection of bacteria or spores.

Since the new sensors are free-standing and have no wires or mechanical connections, they are easily adapted for use in flowing liquids without needing complex circuits or mechanical structures.

2. Principle of operation

The operating principle of this magnetoelastic sensor is based on the phenomenon of magnetoelasticity. Simply stated, upon the application of a magnetic field, the material undergoes a corresponding shape change due to its internal magnetic moments, i.e., it gets longer, wider, thicker based on the magnetic direction [11,12].

Briefly, the platform of this sensor is a magnetoelastic material which has a characteristic resonance frequency that is a function

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* Corresponding author.
E-mail address: shenwen@auburn.edu (W. Shen).

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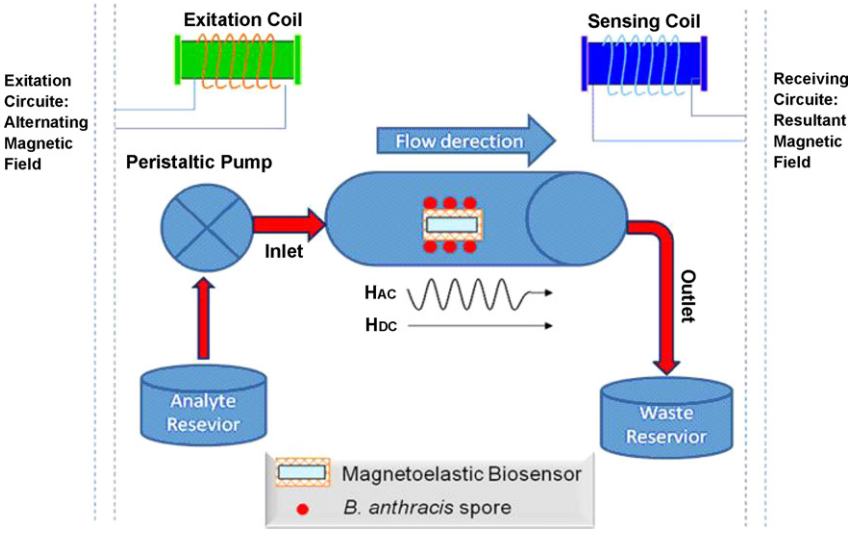

Fig. 1. Schematic of the detection system.

of its material properties [13], shape, physical dimensions [14] and mass of the material [15]. If an alternating magnetic field is applied with a frequency that matches this characteristic resonance frequency, a maximum shape change occurs. This shape change can be sensed by an external frequency monitoring device as explained later in this paper.

When temperature and humidity are constant, a small uniform mass loading on the sensor surface will cause a shift of the resonance frequency by an amount given by [15]

$$\Delta f = -\frac{f}{2}\frac{\Delta m}{M} \quad (1)$$

where f, Δf, M and Δm represent initial resonance frequency of the sensor, the shift in the resonance frequency, initial mass and change in mass of the sensor, respectively. If the increase in mass (Δm) is caused by a target biological analyte attaching to the sensor surface, the resonance frequency will decrease by a corresponding amount (Δf).

Previous research tests in our lab have been done to verify the above equation. The results of these tests are given in the results and discussion.

3. Detection system design and experimental methods

3.1. Sensor platform

A sensor consists of the basic platform plus the bio-recognition element material used to make it sensitive for a specific bacteria or spore.

The magnetoelastic material used for making the sensor platform was Metglas® 2826 MB alloy from Honeywell Inc. This alloy has a saturation magnetostriction of 12 ppm [16]. The basic sensor platform consists of a magnetoelastic device with a thin layer of gold on the surface. The sensors used in this study are free-standing rectangular pieces of 1000 μm × 200 μm × 15 μm in dimensions. The gold layer (100 nm thick) was used to protect the magnetoelastic material from corrosion and at the same time to provide a bioactive surface to which the biological probe may be easily attached. The detailed design and construction of the magnetoelastic sensor platform was published by Lakshmanan et al. [17] and Guntupalli et al. [18].

3.2. Target pathogen and bio-recognition probe selection

After the platform is completed, it is coated with a bio-recognition element. The bio-recognition element used on the magnetoelastic platform was filamentous phage, clone JRB7, derived from a landscape f8/8 phage library developed by the Department of Pathobiology at Auburn University [5,19]. This phage was designed to be a specific probe for *B. anthracis* spores while ignoring other spores. The JRB7 phage was immobilized onto the gold surface of the magnetoelastic sensor platform by immersing the gold-coated sensor platform particles in a JRB7 phage suspension of 10^{12} vir/ml for 1 h. The sensors were then washed with distilled water multiple times and dried in air. Once the phage is immobilized to the sensor surface it serves as the bio-recognition probe for *B. anthracis* spores.

3.3. Signal processing unit and fluid unit design

3.3.1. Detection system

The basic structure of the detection system for our magnetoelastic sensors is presented in Fig. 1. The detection system consists of two main parts; the signal processing section and the fluid flow section. The signal processing section is used for measuring resonance frequency of the sensors. Coils are used for applying a magnetic field to the sensor and for receiving signals from the sensor. By measuring the signal amplitude at each applied frequency, maximum amplitude is measured at the resonance frequency. In our case we monitor the difference between the applied signal and the sensed signal to give a negative going peak.

3.3.2. Fluid unit

The fluid flow section consisted of small reservoir to hold the test solution and another to hold the discard solution. Between the reservoirs is a glass tube containing the sensor and a peristaltic pump used to provide a controlled flow rate. Around the glass tube containing the sensor is a coil set used for applying the magnetic field and sensing the frequency. The reservoirs, pump and sensor tube were connected with flexible tubes to complete the path from start to finish. The sensing coil was connected to an analyzer to sense the resonance frequency change of the sensor as spores accumulated on its surface, adding mass to the sensor. The analyzer also provided a swept frequency signal to the excitation coil to provide