Magnetoelastic Biosentinels for the Capture and Detection of Low-Concentration Pathogens in Liquid

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Abstract—This paper investigates phage-coated magnetoelastic (ME) biosentinels that capture and detect low-concentration pathogenic bacteria in stagnant liquid. These biosentinels are composed of a freestanding ME resonator platform coated with a landscape phage that specifically binds with the pathogens of interest. These biosentinels can be moved through a liquid by externally applied magnetic fields. When a time-varying magnetic field is applied, the ME biosentinels can be placed into mechanical resonance by magnetostriction. As soon as the biosentinels bind with the target pathogen through the phage-based biomolecular recognition, a change in the biosentinel’s resonant frequency occurs, and thereby the presence of the target pathogen can be detected. Detection of Bacillus anthracis spores under stagnant flow conditions was demonstrated.

Keywords – biosentinel; phage; detection; pathogen

I. INTRODUCTION

For centuries, humankind has attempted to mimic the designs of Nature to develop new engineering materials and systems. The human blood system is an excellent example of one of Nature’s amazing creations that inspires us in this work. The human blood contains many components that work synergetically to keep us healthy. As part of the immune system, white blood cells are the main defensive mechanism against pathogenic invaders. There are a variety of white blood cell types (neutrophil, eosinophil, lymphocytes, etc.) that target different pathogens. This capability will serve as a model for bio-inspired sentinels for the capture and detection of invasive pathogens. In this work, bio-inspired sentinels (biosentinels) were developed for the capture and detection of invasive pathogens in liquid analytes. Potential short-term applications include the capture and detection of bacteria in liquid food products such as juices and milk. As an initial model study, detection of Bacillus anthracis spores, a Category A bioterrorism agent, under stagnant flow conditions was investigated.

II. MATERIALS AND METHODS

A. JRB7 Phage and Bacillus anthracis Sterne Spores

Suspensions of JRB7 phage (5 × 10¹¹ virions/ml in a PBS buffer, pH 7.2) and Bacillus anthracis Sterne spores (5 × 10⁶ spores/ml in sterile distilled water) were kindly provided by Dr. James Barbaree’s group at Auburn University. The Sterne strain of B. anthracis is an attenuated strain that is incapable of causing anthrax infection in humans and, thus, convenient for safe laboratory experiments. Yet, all antigenic markers on a Sterne spore are common with those on a pathogenic spore. Hence, the binding characteristics of the affinity-selected JRB7 phage to the Sterne and pathogenic spores are expected to be identical. The concentrated spore suspension was diluted with sterile distilled water as desired prior to use.

B. Fabrication of Biosentinel Platforms

Freestanding, strip-shaped, gold-coated biosentinel platforms with a size of 200 μm × 40 μm × 4 μm were batch-fabricated using the sputtering-based method established previously [1]. These biosentinel platforms are made of an amorphous, ferromagnetic alloy of Fe₇₉B₂₁. The fabricated platforms were then annealed at 220 °C for 2 hours in vacuum in order to relieve residual internal stresses and minimize the effects of any surface defects from the fabrication processes.

C. Immobilization of JRB7 Phage

The annealed biosentinel platforms were individually immersed in 330 μl of the phage suspension in a PCR tube. By rotating the tubes at 8 rpm for 1 hour, the phage was allowed to uniformly attach to platform surfaces via physical adsorption. Finally, these phage-immobilized biosentinels were thoroughly rinsed with sterile distilled water to remove any PBS buffer components and loosely attached phage particles from the platform surfaces.

D. Surface Blocking

In order to reduce non-specific adsorption of spores on biosentinel surfaces, surface blocking with bovine serum albumin (BSA) was performed. The prepared biosentinels were individually immersed in a 330-μl solution of BSA (0.1 % w/v in sterile distilled water) in a PCR tube. After 40 min of tube rotation at 8 rpm, the biosentinels were collected and thoroughly rinsed with sterile distilled water to be ready for use. Control biosentinels, which are not immobilized with JRB7 phage but only surface-blocked with BSA, were also prepared and used as controls for background correction.

E. Orientation and Translation Control of the Biosentinel by External Magnetic Fields

Magnetic fields can be used to orient and propel magnetic bodies in a controlled manner. When a body with internal magnetization (M) is misaligned with an external magnetic field (H), a torque will arise to eliminate the misalignment. In other words, the body will rotate to align its own M-field with

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the external $H$-field. When a gradient exists in the $H$-field, a force is generated upon the body that can result in translational motion. Based on these principles, our biosentinels (ferromagnetic bodies) can be moved through a liquid to capture and concentrate the pathogens of interest.

**F. Principle of Detection**

Biosentinels are made of a ferromagnetic alloy (Fe$_{79}$B$_{21}$) with magnetoelastic properties [2]. Because of its magnetoelastic properties, the biosentinel can be placed into mechanical resonance when subjected to an externally applied magnetic field that alternates at the right frequency. For a freestanding, strip-shaped biosentinel, the fundamental resonant frequency of longitudinal vibration, $f$, can be calculated by [3]

$$ f = \frac{1}{2L} \sqrt{\frac{E}{\rho(1-\nu)}} $$

(1)

where $L$, $E$, $\rho$, and $\nu$ denote the length, elastic modulus, density, and Poisson’s ratio of the biosentinel, respectively. When the biosentinel and target pathogen (*B. anthracis* spore) come into contact with each other, the phage that is immobilized on the biosentinel binds with the pathogen, thereby increasing the total mass of the biosentinel. This change in mass, $\Delta m$, causes a corresponding decrease in the resonant frequency given in Eq. 1. The resultant resonant frequency change, $\Delta f$, can then be approximated as [4 – 7]

$$ \Delta f \approx -\frac{\Delta m}{4L^2W^2T} \sqrt{\frac{E}{\rho^3(1-\nu)}} $$

(2)

where $W$ and $T$ are the width and thickness of the biosentinel, respectively. Equation 2 describes that the frequency change is largely dependent of the size of the biosentinel and inversely proportional to $L^2WT$.

**G. Resonant Frequency Measurement**

For measurement of the resonant frequency, the biosentinels were individually placed into the center of a copper solenoid coil that is connected to a network analyzer (HP/Agilent 8751A from Agilent Technologies, Inc.), operating in the $S_{11}$ reflection mode (Figure 1) [8].

![Figure 1. Frequency measurement setup.](image)

An incident AC signal was applied across the coil to magnetically excite the longitudinal vibration of the biosentinel, and the resultant reflected signal was compared with the incident signal over a proper range of frequencies. Finally, the resonant frequency of the biosentinel was determined to be the frequency at which the largest change in normalized $S_{11}$ occurs, due to the mechanical resonance of the biosentinel. To enhance the magnitude of the resonant peak, a proper bias magnetic field was also applied to the biosentinel with a bar magnet.

**H. Fabrication of a Microfluidic Cell Sorter**

A microfluidic cell sorter (Figure 2) was fabricated of (poly) dimethylsiloxane (PDMS) by multi-layer soft lithography process. This cell sorter consists of four fluidic channels (blue channels) and one central chamber. Control channels (red channels) are located right above the fluidic channels. By pressurizing the control channels, the flexible PDMS membrane between the control and fluidic channels deflects downward to separate the central chamber from the fluidic channels (i.e., closed microfluidic valves). *B. anthracis* spores were introduced to the central chamber through a fluidic channel, and a desired number of spores can be isolated by closing all the valves. In this work, both a biosentinel and spores were captured in the closed central chamber. The biosentinel was then moved through the liquid by external magnetic fields to capture and detect spores under stagnant flow conditions.

![Figure 2. Microfluidic cell sorter: (a) a three-dimensional view of the whole chip on a slotted glass slide and (b) a close-up view of the key elements of the chip.](image)
III. RESULTS AND DISCUSSION

A. Rotation and Translation of Biosentinels

Figure 3 demonstrates the rotation and translation of biosentinels in water. Randomly oriented biosentinels (Figure 3a) can be aligned with an externally applied magnetic field (Figure 3b) due to the torque generated. In addition, these biosentinels can be translated in the water by the gradient existing in the external magnetic field.

B. Detection of B. anthracis Spores

Based on the above result, external magnetic fields were used to introduce a 200 µm long biosentinel into the central chamber of the microfluidic cell sorter (Figure 4a) that had been filled with a desired concentration of spores (Figure 4b). All the four valves were then closed, creating a stagnant flow condition in the chamber. Since spores are dormant cells and cannot actively move unlike germinated cells, it is difficult to efficiently capture these spores under these flow conditions. By magnetically controlling the position of the biosentinels, efficient capture was realized. As can be seen in Figure 4b, fluorescent labeled spores were successfully bound to the surface of the biosentinel within a short period of time (~ 10 minutes).

Figure 4. (a) Optical micrograph of the microfluidic cell sorter. (b) Fluorescent micrograph showing B. anthracis spores are bound to a 200 µm × 40 µm × 4 µm biosentinel. The chamber was outlined with a yellow solid line for better visualization.

Figure 5 shows resonant frequency changes of measurement and control biosentinels (with and without phage) as a function of bound spores. Down to 10^6 spores
were successfully detected in 10 minutes. Hence, proof-in-principle has been demonstrated.

Figure 5. Responses of biosentinels (200 µm × 40 µm × 4 µm) to various numbers of spores.

IV. CONCLUSIONS

In this work, we attempted to mimic white blood cells by the creation of engineered magnetoelastic biosentinels that seek out, capture, and detect the pathogen of interest. By using external magnetic fields, the biosentinels were successfully moved through a liquid, and detection of spores under stagnant flow conditions was demonstrated.

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