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Photothermal spectroscopy of *Bacillus anthracis* and *Bacillus cereus* with microcantilevers

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Abstract

Microcalorimetric optical and infrared spectroscopy is a method of determining the spectral absorption of small quantities of materials over a wide range of incident wavelengths. In this paper, the first spectroscopic results for microcantilevers coated with *Bacillus anthracis* (BA) are presented. These results, for *B. anthracis* from 2.5 to 14.5 μm, are compared with results from microcantilevers coated with *Bacillus cereus* (BC) and standard spectroscopic absorption data. The results demonstrate strong correlation between the deflection measurements and the reference spectroscopic absorption peaks. An advantage of this microcantilever-based method over traditional spectroscopy is that much smaller amounts of material (nanogram quantities) can be detected in comparison with the milligram amounts needed for standard methods. Another advantage is that the complete system can be relatively small without sacrificing spectral resolution.

Keywords: Microcantilever; Sensor; Photothermal

1. Introduction

As the threat from terrorist groups increases, the necessity of detecting small amounts of biological and chemical species becomes increasingly important. One of the more infamous materials used in a recent terrorist threat [1] has been *Bacillus anthracis* (BA), more commonly know as "Anthrax". BA is a rod shaped aerobic spore-forming bacterium [2] that causes a biological infection in humans. Dangerous amounts of the spores can be spread over a large area and amounts damaging to human health (10^5 BA spores [3]) can be inhaled or ingested. The other material tested in this study is *Bacillus cereus* (BC), a species commonly used as a simulant for BA. BC has many chemical and physical properties similar to BA without the devastating health effects.

Recently, the microcantilever has attracted much attention due to its versatility as a sensor platform for physical, chemical, and biological sensing [4–6]. Compounds are detected by monitoring changes in the resonant frequency induced by adsorbate mass loading, or by quasi-static deflection (bending) of the microcantilever when molecular adsorption is confined to a single side [7]. Adsorption changes the mass and surface free energy of the microcantilever. It has also been shown that bi-material microcantilevers are extremely sensitive to thermal radiation and extremely sensitive room temperature infrared sensors have been demonstrated with metal-coated cantilevers. Exposing an adsorbate-covered cantilever to regions of wavelengths where the adsorbate molecules have strong absorption peaks, makes the cantilever bend [8]. The mechanical bending of the cantilever closely resembles the absorption spectra of the adsorbate molecules. This photothermal spectroscopy with microcantilevers [3,9–11] opens the door for the detection of very small amounts of BA as well as many other chemical and biological species. An advantage of this microcantilever method over traditional spectroscopy is that much smaller amounts of material (nanogram quantities) are required. Traditional infrared spectroscopic methods require milligram amounts of the compound to be studied. Another advantage is that microcantilevers arrays can be produced in silicon and a

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complete analytical system can thus be provided that is relatively small and economical.

The photothermal signals that are observed correspond in general to traditional infrared absorption spectra. Heat from the decay of vibrational and rotational states is transferred to the microcantilever causing it to bend due to the bi-material effect (in which one material expands more than another). The microcantilevers consist of a layered Au and silicon nitride structure. Since Au has a larger thermal expansion coefficient than silicon nitride, the microcantilever deflects towards the silicon nitride side when it is heated.

This method is not restricted to the infrared region of the wavelength spectrum but can also be used in the visible and ultraviolet regions as long as the sample being studied absorbs the radiant energy and reemits it as heat. Samples can be studied from the UV–vis region of the spectrum all the way into the far-infrared region of the spectrum. Unfortunately, in the 0.3–2 μm region of the wavelength spectrum there are not many characteristic peaks for the BA or BC spores. A small absorption peak can be observed around 280 nm. In the visible region other factors can come into play and thin film optical interference effects can be observed [12].

Photothermal spectroscopic results are presented here for microcantilevers coated with BC and BA and illuminated with wavelengths from 2.5 to 14.5 μm. The results are compared with standard spectroscopic absorption data. The data demonstrate strong correlation between the photothermal deflections and the standard spectroscopic absorption peaks of BC and BA.

2. Experimental

2.1. Biological material

In this experiment, microcantilevers coated with BC and BA spores are used as experimental samples. BC ATCC-7064 was purchased from American Type Culture Collection (Manassas, VA) and was grown in a medium consisting of 24.00 g of Nutrient Broth (Difco), 7.5 g of KCl, 0.5 ml of 1-M CaCl2, 0.25 ml of 0.01-M FeCl3, 0.5 ml of 0.01-M MgSO4, and 0.5 ml of 50% glucose L. All the ingredients except the Nutrient Broth and KCl were filter sterilized and added after autoclaving. A half milliliter of overnight preculture was inoculated into 250 ml of the medium in a 1000-ml flask and incubated at 37 °C in a shaker for four days. Spores were harvested by foam flotation, and the richness of the spores was confirmed by optical microscopy. The harvested spores were washed with sterile deionized distilled water several times and lyophilized for use in our experiments [13].

A non-infectious sample of BA Ames spores was obtained from the U.S. Army Dugway Proving Ground, Utah. The sample vials containing 1 mg/ml suspensions of the spores in water were shipped to us frozen in dry ice. The spores had been killed using a total dose of 5.0×10^6 rad of cobalt (gamma) irradiation over 347 min. The sterility of the spores was confirmed on 23 January 2003 at the Life Sciences Test Facility, Life Sciences Division, Dugway Proving Ground, Utah. Confirmation of the nonviability of the spores was obtained by placing a subpopulation of the spores in a broth culture (24 and 72 h at 37 °C in tryptic soy broth) and subsequent subculture to trypicase soy agar plates for 24- and 48-h cultures [14].

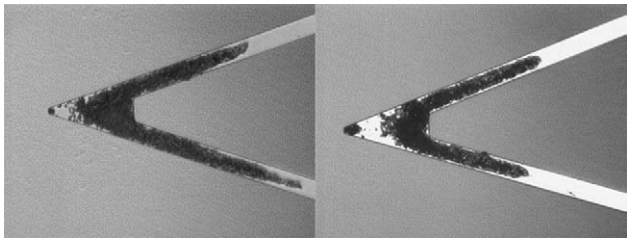

Fig. 1. *Bacillus anthracis* coating on the SiN side of the microcantilever (left) and on the Au side (right). The cantilever is 300 μm long.

2.2. Sample preparation

The coatings are produced by physically dipping the microcantilever probes into a 10 μl suspension that consists of water and spores. As the water evaporates a thin layer of spores is deposited over both sides of the microcantilevers surface. Fig. 1 shows an optical micrograph of the coating for a BA sample. All of the sample coatings in this experiment looked similar to the microcantilevers shown in Fig. 1. Sublimation, using a heating source to evaporate material onto the surface, can also be used to deposit material, but the dipping method provides better results and allows the use of smaller amounts of sample material. Dipping the cantilevers also avoids any damage to the spores that could occur during the heating process.

In addition to visual inspection to confirm coating presence, the mass on the cantilevers, δm, is calculated using the relationship

$$\frac{\delta m}{m} = \left[\left(\frac{\nu_o}{\nu_m}\right)^2 - 1\right]$$

where ν_o is the resonance frequency of the cantilever before coating, ν_m is the frequency after the addition of the spores, and m is the original mass of the cantilever [15]. The combination of visual inspection and frequency shift (mass change) demonstrates that the material is attached to the microcantilever.

2.3. Photothermal setup

Commercially available 300 μm long, V-shaped, SiN microcantilevers [16] that consist of a Silicon Nitride (SiN)–Chromium (Cr)–Gold (Au) layered structure are used as the sensor platform. The cantilevers are illuminated with monochromatic infrared radiation from a custom-built filter-based monochromator. A schematic diagram of the experi-