A microcantilever-based pathogen detector

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ABSTRACT

The ability to detect small amounts of materials, especially bacterial organisms, is important for medical diagnostics and national security issues. Engineered micro-mechanical systems provide one approach for constructing multifunctional, highly sensitive, real-time, immunospecific biological detectors.

We present qualitative detection of specific *Salmonella* strains using a functionalized silicon nitride microcantilever. Detection is achieved due to a change in the surface stress on the cantilever surface in-situ upon binding of a small number of becteria. Scanning electron micrographs indicate that less than 25 adsorbed bacteria are required for detection.

Keywords: Microfabrication; Cantilever; Biosensor; Immunosensor; Surface functionalization

1 INTRODUCTION

The atomic-force microscope (AFM) cantilever is not only crucially important for scanning probe microscopy; it has proven to be indispensable in many surface science applications. In addition AFM micromachined cantilevers are an important component in many micromechanical sensors. Recent experiments have used AFM cantilevers as versatile sensors to distinguish between DNA oligonucleotides,[1] to measure pH changes,[2] and to measure the surface stress associated with molecular adsorption[3] or absorption.[4] In many of these applications, the deflection of the cantilever is driven by the build up of surface stress as its surface is modified. The surfaces stress associated with a cantilevers deflection can be predicted by Stoney's formula and has been investigated in detail.[5]

An alternative approach for cantilever-based detection is based on dynamic interrogation using resonant shift. In this approach, additional mass loading results in a change of the resonant frequency of the cantilever system. This technique has been successful in the detection of single microbes of *E. coli*.[6] Although resonant frequency shift yields the highest sensitivity this method has many drawbacks. For example, low quality factor in liquids and the position of the microbe along the cantilever affects the frequency shift. The low quality factor precludes real-time use in liquids due to the broadening of the resonant peak. Therefore experiments thus far have focused on measuring the resonance shift in air (or vacuum) after exposure to the matrix.

Here we report a real time method for the detection of whole bacterial organisms based on the change in surface stress of the cantilever.

2 EXPERIMENTAL

2.1 Experimental Setup

Experiments were performed in a Digital Instruments Mutimode Instrument (Santa Barbara, CA). A low-power He-Ne laser (<3 mW power) is focused onto the tip of the cantilever. The laser beam reflected off the cantilever is directed into a position-sensitive diode (PSD) that can detect the vertical position of a laser beam. A fluid cell—commercially available from Digital Instruments within which the cantilever is mounted, forms a 100 μL cavity. V shaped cantilevers (TM Microscopes, Sunnyvale, CA) were used in all experiments. Results presented were collected on the A cantilever which is 180 μm long and 18 μm wide. The nominal spring constant of the cantilevers is 0.05 N/m. Thermal effects were neglected in these experiments, however a temperature control system can potentially maintain a set temperature of ± 0.05 °C.

2.2 Reagents

Dithiobis(sulfosuccinimidylpropionate) (DTSSP), obtained from Pierce Chemical Company (Rockford, IL), is a water-soluble, homobifunctional N-hydroxysuccimide (NHS) ester. It is thiol-cleavable and widely used for conjugating radiolabeled ligands to cell surface receptors. Anti-salmonella antibody (ASA) was supplied from the United States Department of Agriculture (Albany, CA) and used as supplied. Affinity-purified BSA was ordered from Pierce Chemical Company. Other chemicals were purchased from Sigma-Aldrich (St. Louis, MO). Two specific inactivated strains of Salmonella were investigated (Heidelberg and Typherium). Both strains along with the

monoclonal antibodies to the specific strains were supplied by the USDA.

2.3 Cantilever Functionalization

The cantilevers were supplied by the manufacturer with a gold coating for reflectivity. To remove contaminates from the surfaces the cantilevers were cleaned for 10-15 minutes in SC-1 solution (1:1:5 – NH4OH:HOOH:H2O) at 80 °C. The cantilevers were subsequently rinsed with deionized water for 10 min. This process was done immediately before the experiments. The fluid cell and glass slide were cleaned using standard detergent for glassware and rinsed with large amounts of deionized water for about 10 min.

DTSSP was dissolved in 5 mM sodium citrate buffer (pH = 5.0) at a concentration of 1.5 mM just before use since DTSSP is moisture-sensitive. Cantilevers immersed in this solution for about 2 h at room temperature results in strong adherence of DTSSP to the gold surface by a disulfide linkage. After derivatizing with DTSSP, the cantilevers were rinsed with 20 mM sodium phosphate buffer, 0.15 M NaCl and pH 7.5 (PBS) for 5 min and then immersed in ASA solution for at least 5 h at room temperature. The cantilever was washed by 1 mg/ml BSA solution in PBS (BSA/PBS) thrice for about 10 min and stored in BSA–PBS solution overnight at room temperature. The BSA treatment 'caps' any exposed areas of the cantilever to prohibit non-specific binding.

2.4 Procedure

The functionalized cantilever was mounted in the fluid cell and equilibrated in BSA–PBS solution until a stable baseline of cantilever deflection was obtained (usually around 1 h). The control (such as BSA–PBS or a nonspecific strain of salmonella) was then injected into the fluid cell and cantilever deflection was monitored in situ. All the experiments were carried out at room temperature. Although there is some thermal drift in the experiment this did not affect the binding or the experimental outcome. Because there was no flow through the fluid chamber, the reaction happened in a static environment. The deflection was monitored in real-time using a computer equipped with a DAC card and custom LabView software to capture the signal from the photodetector.

3 RESULTS/DISCUSSION

Figure 1 shows a typical result that we obtained for two different strains of *Salmonella*. For both runs the cantilever was functionalized with the anti-*S. Heidb.* antibody. The large spike at the beginning of each run is due to the injection of Salmonella solution creating a hydrodynamic pulse and a subsequent deflection of the cantilever. The first injection was the *S. Typh.* strain at a concentration of 100 colony forming units (cfu)/ml. No deflection of the cantilever beyond the thermal equilibrium is detected over a period of hours. To remove the residual *S. Typh.* A PBS buffer solution is flushed through the cell. The effect on the cantilever is similar to that of the *S. Typh.* injection showing no bending beyond the thermal effects. In contrast, subsequent injection of *S.Heidb.* causes a large and permanent deflection of the cantilever within 20 seconds.

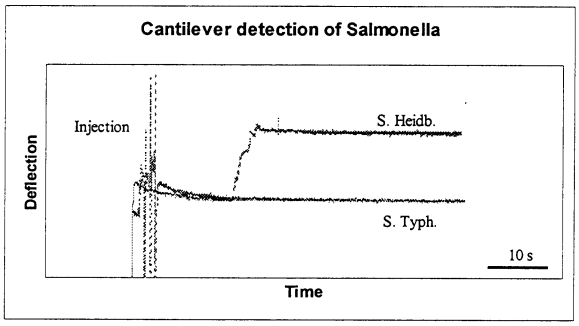

Figure 1: Typical experiment showing the detection of a specific strain of Salmonella.

Determining the detection limits for this technique is complex since it is difficult to quantify the effective concentration of the bacteria exposed to the sensor. We are not using a flow cell setup, therefore the concentration of the bacteria in the fluid cell changes as more bacteria bind to the cantilever. The diffusion of Salmonella bacteria is slow making concentration equilibrium difficult. Still, we ran experiments by varying the concentration of the *Salmonella* which indicate the cantilever deflection signal increased roughly proportional to the bacteria concentration (Figure 2).

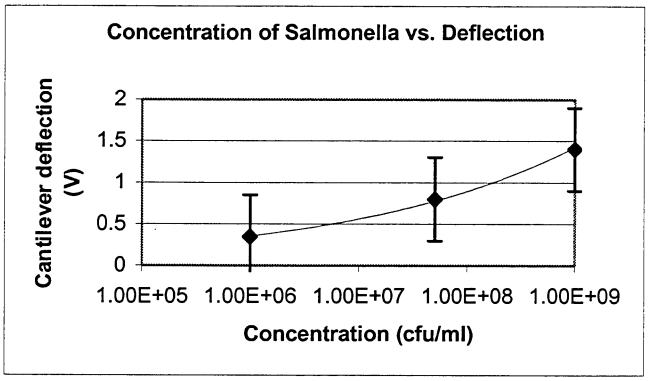

Figure 2: Dependence of concentration on cantilever deflection for *S. Heidb.*

Fortunately, the large size of the *Salmonella* bacteria yields a convenient method to quantify the binding by counting the number of bacteria attached to the cantilever surface. The bacteria were counted by imaging the cantilever after experiments were complete in a field emission scanning electron microscope. Images collected on the unfunctionalized side of the cantilever showed no binding of bacteria. The functionalized side produced clear evidence of bacteria as shown in figure 3 when the complimentary strain of Salmonella was exposed to the antibody specific for that strain. Binding of the non-complimentary strain was never observed on the cantilever.

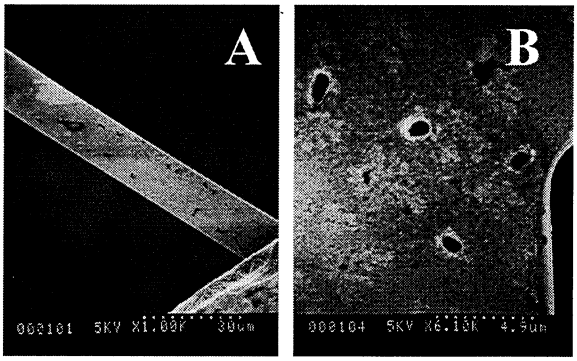

Figure 3: A: SEM micrograph showing *Salmonella* attached to the cantilever surface functionalized with the antibody. No Salmonella was observed in the unfunctionalized areas. B: Close up of *S Heidb.* on cantilever.

Figure 4 shows a graph of the number of bacteria adsorbed on the cantilever surface in relationship to the observed deflection. The cantilevers are the same as used in Figure 2. Clearly, the number of bacteria provides an interesting quantification for the performance of the cantilever detector. The large error bars are the result of a number of factors which are not controllable in our experiments. For example, bacteria absorbed at the base of the cantilever contribute to the deflection more than those at the end. We also used different cantilevers with differing spring constants for each run. Still, the trend line allows us to estimate the detection limit of this technique. The smallest number of bacteria we were able to count on a cantilever, which yielded a discernable deflection, was about 25. Interestingly enough, this quantity is roughly the toxicity limit for some forms of *Salmonella* poisoning. Therefore, we believe that our technique has the sensitivity required for practical applications.

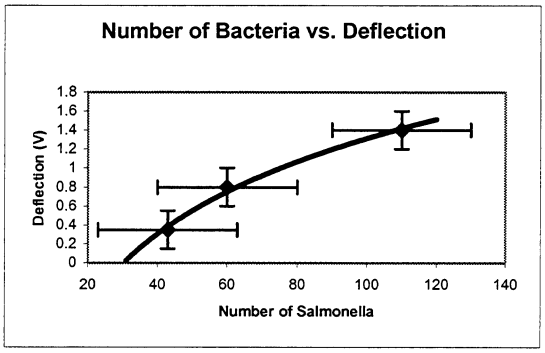

Figure 4: Graph showing the dependence of cantilever deflection on the number of bacteria counted.

4 CONCLUSIONS

We have demonstrated the use of a microcantilever sensor for the detection of whole pathogenic organisms. This system provides numerous advantages such as: high sensitivity and selectivity, low analyte volume, real time detection in fluids and/or air, possibility to create portable and implantable devices. In addition, the fabrication process is easily adapted to: batch fabrication, multiplexing (arrays of cantilevers can be fabricated for detection of multiple pathogens), and integration with other semiconductior processes to produce 'on chip' complete sensing devices.

5 ACKNOWLEDGEMENTS

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