A silicon microcantilever sensor was developed for the detection of Escherichia coli O157:H7. The microcantilever was modified by anti-E. coli O157:H7 antibodies on the silicon surface of the cantilever. When the aquaria E. coli O157:H7 positive sample is injected into the fluid cell where the microcantilever is held, the microcantilever bends upon the recognition of the E. coli O157: H7 antigen by the antibodies on the surface of the microcantilever. A negative control sample that does not contain E. coli O157:H7 antigen did not cause any bending of the microcantilever. The detection limit of the sensor was \(1 \times 10^6\) cfu/mL when the assay time was < 2 h.

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In N$_2$ saturated cantilever was immersed into a 10% succinic anhydride solution for 6 h, followed by rinsing the microcantilever in H$_2$O. Then, the cantilever was immersed into a 10% succinic anhydride solution in N,N-dimethylformamide (DMF) for 6 h, followed by a thorough water rinsing. The microcantilever was then activated by a 0.05 mM MES (4-morpholinepropanesulfonic acid) buffer solution containing 100 mg/mL of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and 100 mg/mL of N-hydroxysuccinimide (NHS) (pH = 6.8) for 30 min at room temperature. At last, the antibodies were covalently immobilized on the microcantilever surface by incubating the microcantilever in a 5 µg/mL antibody solution in 0.1 M PBS (pH 7.3) for 3 h.

The deflection experiments were performed in a quartz flow glass cell (Digital Instruments, CA). The V-shape microcantilever was placed in the flow cell and equilibrated with PBS buffer solution. Once a steady baseline was attained, experimental solutions containing different concentrations of E. coli O157:H7 were injected directly into the liquid cell. To eliminate thermomechanical motion of the silicon cantilever caused by temperature fluctuations, we mounted the fluid cell on thermoelectric coolers so that the temperature of the fluid cell could be controlled to 20 ± 0.2°C. Microcantilever deflection measurements were determined using the optical beam deflection method.

Figure 1 shows the deflection of an antibody coated cantilever as a function of time for 5 × 10$^6$ cfu/mL E. coli in 0.1 M PBS buffer (pH = 7.3). It was observed that the microcantilever immediately bent down after exposure to E. coli solution, but the bending did not reach its maximum even 5 h after the injection, suggesting that the capturing of E. coli by the antibodies on the microcantilever surface was not complete. This may be due to the steric effect caused by the big size of the cells or fragments. Furthermore, when the E. coli solution was replaced by the buffer solution, the cantilever bending remained at that level and did not bend back to its original position (figure not shown), which ruled out the possibility of physical absorption of antibodies on the cantilever surface.

It was anticipated that the Langmuir adsorption model could be used to describe the absorption of E. Coli cells on the antibody covered surface. The rate of formation of a fraction of a monolayer, θ, is proportional to the concentration of the reacting species in solution and to the fraction of the surface remaining free of sorbant, 1–θ. Thus, the cantilever bending vs. the time follows the relationship

$$\delta \propto 1 - \exp(-kt)$$  \hspace{1cm} (1)

Where δ is the surface stress, k is the reaction rate, and t is the time. The k was calculated to be 2.3 × 10$^{-4}$ s$^{-1}$ using a non-linear curve-fitting method to fit the observed experimental data. The fitted curve (dashed line) is shown in Fig. 1.

A control experiment was performed with an antibody conjugated microcantilever to E. coli cell line JM105 (KPL, Gaithersburg, MD), a negative control sample that contains no E. coli O157:H7 antigen. All other experimental conditions were the same. No deflection of the cantilever was observed upon exposure to a 10$^7$ cfu/mL solution of JM105, as shown in Fig. 1. Another control experiment was performed with an unmodified microcantilever to E. coli positive control and no deflection was observed upon exposure to the E. coli.

It has been observed that, 1 h after exposure to a 10$^7$ cfu/mL solution of E. coli O157:H7, an antibody-modified microcantilever was covered by E. coli cells or cell fragments, while no cell was absorbed on the bare gold microcantilever (Fig. 2). Since the E. coli cells were killed by heat that resulted in cell lysis, the positive control sample was not whole E. coli cell, but a random mix of cellular fragments from the bacteria.

The deflection amplitude of an antibody modified microcantilever 2 h after exposure to E. coli versus the concentration of E. coli (Fig. 3) shows that the microcantilever can be used for the detection of E. coli with a detection limit of 10$^6$ cfu/mL.

An anti E. coli O157:H7 antibody-immobilized microcantilever has been demonstrated as a novel biosensor for the detection of E.coli O157:H7 with a detection limit of 1 × 10$^6$ cfu/mL in 2 h after exposure to E. coli at room temperature. These results suggested that other pathogens, such as biological warfare agents, can be detected by using microcantilever sensor technology. The sensitivity of this sensor, however, was less than expected since most of other microcantilever sensors developed were extremely sensitive. However, the detection limit can be improved by using improved micro/nanocantilevers, such as using microcantilevers made of less rigid materials. We are moving toward these areas to improve the sensitivity of microcantilever sensors.

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Fig. 3 Deflection amplitudes for antibody modified microcantilevers 2 h after exposure to E. coli vs. the concentration of E. coli O157:H7 positive control.

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