An Anti E. Coli O157:H7 Antibody-Immobilized Microcantilever for the Detection of Escherichia Coli (E. coli)

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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 x 10⁶ cfu/mL when the assay time was < 2 h.

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Enterohemorrhagic Escherichia coli serotype O157:H7, first recognized in 1982 in the United States, is an epidemiologically significant cause of food borne disease worldwide. E. coli O157:H7 can readily contaminate ground beef, raw milk, poultry products, fresh apple cider, cold sandwiches, vegetables, and drinking water supplies. It can be transmitted efficiently from person to person not only via contaminated food, but also by sharing contaminated facilities. The largest reported outbreaks, which involved 6500 cases and 11 fatalities, occurred in Japan in May and July of 1996. The largest recall of food contamination with E. coli O157:H7 occurred in the summer of 1997 when 25 million pounds of hamburger meat was recalled by a single meat-processing company.²

E. coli O157:H7 has abilities to cause any one or all the illnesses of a devastating disease triad (hemolytic ulcerative colitis, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura) with a low infectious dose (≤ 10 cfu).³ The U.S. Food Safety Inspection Service (FSIS: Athens, Ga.) has established a zero-tolerance threshold for E. coli O157:H7 contamination of raw meat products.⁵ Any level of contamination with E. coli O157:H7 is considered adulterating.

Traditional E. coli strain identification techniques normally take two to three days for selective and confirmative enrichments and up to four days for a final biochemical and serological characterization. A lot of effort has been taken in developing methods to improve the total assay time and sensitivity. One of the most promising approaches is the immunologic recognition method for the specific detection of E. coli O157:H7.⁶ Micro-contact scanning probe microscopy-patterned microarrays of antibodies were able to detect E. coli O157:H7 with a detection limit of 7 x 10⁶ cells/mL.⁷ A membrane filter-covered carbon electrode has demonstrated a detection limit of 5000 bacteria/mL by square wave voltammetry.⁸ Another electrochemical method involves coupling with an immunologic separation; this would detect 10⁵ cfu/mL E. coli in 2 h.⁹ Many other electrochemical,¹⁰ colorimetric,¹¹ potentiometric based sensors,¹²,¹³ polymerase chain reaction (PCR)-based techniques,¹⁴,¹⁵ for E. coli detection have been reported; each has advantages and disadvantages.

Microcantilever-based sensors have received a lot of attention recently. Not only do they provide several orders of higher sensitivity comparing to conventional measurement techniques, but they also have excellent dynamic response in a small package; one has the opportunity to have integration of micromechanical components with on-chip electronic circuitry.¹⁶–²² Basically, microcantilevers enable the detection of three main phenomena: surface stress changes, heat changes and mass changes upon adsorption of binding of analytes on the microcantilever surface. Many chemical or biological interactions involve more than one of these changes. Microcantilevers provide an outstanding platform to detect these interactions. This work presents a microcantilever-based sensor for in situ detection of E. coli O157:H7. The microcantilever was modified by anti-E. coli O157:H7 antibodies to capture the intact E. coli cells or fragments.

Some immunoassay reagents included affinity-purified antibodies to Escherichia coli O157:H7, and positive control of E. coli O157:H7 bacteria was obtained from Kirkegaard & Perry Laboratories (Gaithersburg, MD). The positive control bacteria used in our work were killed by heat. The affinity purified antibody was one that has been purified from the IgG fraction by affinity chromatography to an E. coli O157:H7. A 0.1 M PBS (pH = 7.3) buffer solution was used in all the experiments. The buffer solution was composed of 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na₂HPO₄ and 1.4 mM KH₂PO₄.

We used commercially available silicon microcantilevers (Veeco Instruments, CA) in our experiments. The dimensions of the V-shaped silicon microcantilevers were 180 µm in length, 25 µm in leg width, and 1 µm in thickness. One side of these cantilevers was covered with a thin layer of chromium (3 nm) followed by a 20-nm layer of gold, both deposited by e-beam evaporation.

Microcantilever modification was completed in four steps according to known surface conjugation chemistry.²³ First, a thin film of aminopropyl triethoxysilane (ATS) was formed on the silicon surface by immersing the cantilever into a 1% ATS in an EtOH:H₂O = 95:5 solution at room temperature for 24 h.

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followed by rinsing the microcantilever in H$_2$O. Then, the cantilever was immersed into a 10% succinic anhydride solution in N$_2$ saturated 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 buffer (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 obtained, 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 at 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 $5 \times 10^6$ 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 the capturing of $E. coli$ by the antibodies on the microcantilever surface was not complete. This may 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, $\theta$, is proportional to the concentration of the reacting species in solution and to the fraction of the surface remaining free of sorbant, $1 – \theta$. Thus, the cantilever bending $\delta$ vs. the time follows the relationship:

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

Where $\delta$ is the surface stress, $k$ is the reaction rate, and $t$ is the time. The $k$ was calculated to be $2.3 \times 10^{-4}$ 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 $1 \times 10^7$ 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 $1 \times 10^6$ 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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