Detection of Viability Change of *Escherichia coli* O157:H7 using Surface Plasmon Resonance

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Abstract

For the acute assessment on biological toxicity of wastewater, surface plasmon resonance (SPR) based cell viability detection was performed using gold surface-confined cell as a result of adhesion-modifying chemicals. *Escherichia coli* O157:H7 (*E. coli* O157:H7) was investigated after exposure to EDTA. Cells were immobilized on gold coated slide glass for SPR analysis by the method of cross-linking carboxyl group on the bacterial surface with amine group of poly-L-lysine that had been coupled to the gold surface modified by a self-assembled monolayer of 11-mercaptounde canoic acid (11-(MUA))[1]. Reflective intensity of each flow step was changed with respect to contect of ethylenediaminetetraacetic acid (EDTA) disodium salt and phosphate-buffered saline (PBS) solution. The proposed detection technique can be used for biological toxicity test.

Introduction

A microbial sensing system with the special processing algorithm has been developed for detecting the toxicity or over-nutritivity of physically chemically pretreated wastewater for further biological treatment[2]. Because there is a practical need to obtain as much information as possible about the chemical exposure, it is necessary to not only identify possible toxicant but also obtain assurance that chemical soup we do live in. Microbial based detection technique provides an attractive alternative to conventional analytical methods (high performance liquid chromatography, gas chromatography mass spectroscopy, standard culturing and microscopic examination methods, and enzyme-linked immunosorbent assay-ELISA) which are relatively time consuming and require costly and cumbersome equipment. The use of bacteria-immobilized surface enables us to rapidly assess the biological

toxicity of water. In the present work, the change in the viability of *E. coli* O157:H7 is investigated using surface plasmon resonance[3-5].

Material and Methods

Cell Culture. *E. coli* O157:H7 was cultured in a 250 ml Erenmyer with 100 ml of medium (medium composition: pancreatic digest of casein 10g, NaCl 5g, yeast extract 5g in 1L deionized water, pH 7.0±0.2 at 25℃) at 37℃ with shaking 200 rpm.

Thin film fabrication. Gold coated cover slide glass was prepared using the sputtering. The specification was first Cr 2 nm sputtering and then Au 43 nm sputtering. Gold glasses of 1.8 cm^2 were cleaned with "piranha solution" in 5 minutes at 60℃, first rinsed with ethanol and second with deionizes distilled water, and dried by N_2 gas. Cleaned glasses were coated 11-(MUA) by self-assembly technique in 24 hour. Poly-L-Lysine (0.1% w/v) recoated the treated glasses in 12 hour at room temperature for attaching cells[1,6]. In each step, glasses were cleaned and rinsed with deionized water.

Detection using SPR. For the experiment setup of SPR, a He-Ne laser was used as a light source to make a monochromatic light with a wavelength of 632.8 nm. The p-polarized light beam by the polarizer was used as a reference and the intensity of the reflected beam was measured by photo multiplier tube (PMT) sensor.

Results and Discussion

The change of the SPR curve by adsorbing 11-(MUA), 0.1% w/v Poly-L-Lysine, *E. coli* (OD: 1.23), 1%(wt.) EDTA was showed in Fig. 1. The SPR minimum position was shifted significantly from 43.002°±0.02 to 43.198°±0.03 by the adsorption of 150 mM 11-(MUA) on the Au surface. And, the SPR minimum position was shifted from 43.198°±0.03 to 43.462°±0.03 by binding between amine group of poly-L-lysine and the activated carboxyl group of 11-(MUA). As a result of the change of these minimum angles, immobilization of each step was successful. The SRP minimum degree of the *E. coli* O157:H7-immobilized surface was shifted from 43.462°±0.03 to 43.223°±0.04 by each treatment of 1% wt EDTA. Fig. 2 shows the time-course behavior of reflectance on the surface of the fabricated poly-L-lysine film. For the investigation of immobilization step, a flow cell was construct, equipped with peristaltic pump.

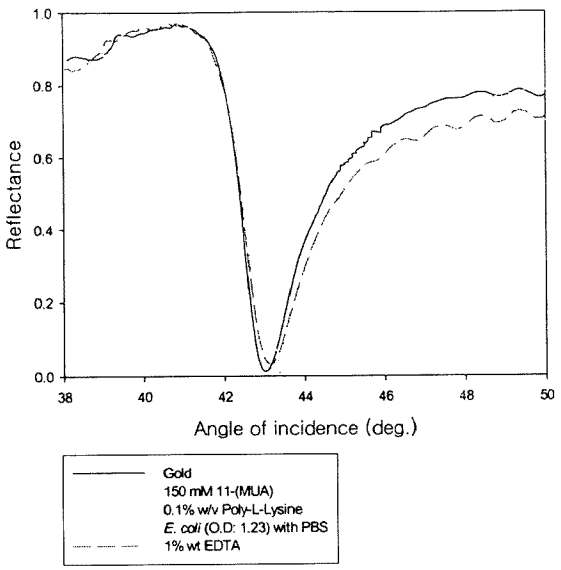

Fig. 1. SPR spectroscopy of bare gold, 150 mM 11-(MUA), 0.1% (w/v) Poly-L-Lysine, *E.coli* O157:H7 (O.D:1.23), 1%(wt.) EDTA

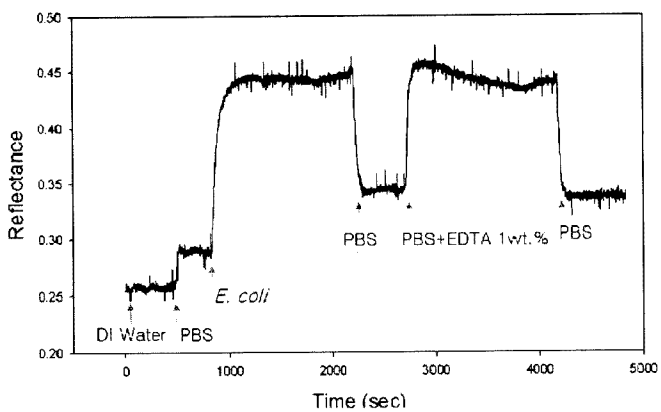

Fig. 2. The reflectance of the flow cell vs. time(sec). The sequence for detection was deionized water, PBS, *E. coli* O157:H7 with medium, PBS, 1% (wt.) EDTA, and PBS.

When the *E. coli* O157:H7 was injected into the flow cell. The reflectance of SPR was significantly increased. After the washing step started using PBS. The reflectivity difference between fabricated poly-L-lysine film and *E. coli* O157:H7 immobilized surface was remarkable.

After the EDTA was injected into the flow cell and washing with PBS was finished, the reflectivity difference was observed, which means the change of *E. coli* O157:H7 viability. The result suggests that this technique can be used for the biological toxicity test and one of the detection method of cell based array.

Acknowledgment

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