J. Microbiol. Biotechnol. (2006), 16(8), 1169–1173

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

© The Korean Society for Microbiology and Biotechnology

Immunosensor for Detection of *Escherichia coli* O157:H7 Using Imaging Ellipsometry

BAE, YOUNG MIN[1], KWANG-WON PARK[1], BYUNG-KEUN OH[1,2], AND JEONG-WOO CHOI[1,2*]

[1]*Department of Chemical and Biomolecular Engineering, Sogang University, Seoul 121-742, Korea*
[2]*Interdisciplinary Program of Integrated Biotechnology, Sogang University, Seoul 121-742, Korea*

Received: December 12, 2003
Accepted: December 29, 2004

Abstract Imaging ellipsometry (IE) for detection of binding of *Escherichia coli* O157:H7 (*E. coli* O157:H7) to an immunosensor is reported. A protein G layer, chemically bound to a self-assembled layer of 11-mercaptoundecanoic acid (11-MUA), was adopted for immobilization of monoclonal antibody against *E. coli* O157:H7 (Mab). The immobilization of antibody was investigated using surface plasmon resonance. To fabricate antibody spots on a gold surface, protein G solution was spotted onto the gold surface modified with an 11-MUA layer, followed by immobilizing Mab on the protein G spot. Ellipsometric images of the protein G spot, the Mab spot, and Mab spots with binding of *E. coli* O157:H7 in various concentrations were acquired using the IE system. The change of mean optical intensity of the Mab spots in the ellipsometric images indicated that the lowest detection limit was 10^3 CFU/ml for *E. coli* O157:H7. Thus, IE can be applied to an immunosensor for detection of *E. coli* O157:H7 as a detection method with the advantages of allowing label-free detection, high sensitivity, and operational simplicity.

Key words: Imaging ellipsometry, *Escherichia coli* O157:H7, self-assembly, immunosensor

The development of a detection method of protein-protein binding on a solid surface is a very important for immunosensors based on antigen-antibody binding. Labeling techniques, in which the binding of protein can be detected after being tagged with a label molecule, such as fluorescein and enzymes, have usually been applied widely as the detection method. Because the labeling techniques have one or two steps for binding of some labeled probes in their protocol, a label-free detection method has advantages as a direct approach for detection of protein-protein binding. Several techniques such as impedance spectroscopy, surface plasmon resonance (SPR), and ellipsometry have been applied to the detection of binding of antigen to an antibody layer immobilized on a solid surface [3, 11]. Especially, SPR, based on the attenuation of surface plasmon generated at an interface between a metal surface and a dielectric layer, has matured to become a versatile tool for studies of protein adsorption kinetics and antibody-based immunosensors. However, because a metal surface, usually gold surface, is used as a substrate for SPR, the immobilization method is confined to the self-assembly between gold and organosulfur materials.

On the other hand, ellipsometry, an optical technique based on measurement of the changes of polarization state of an elliptically polarized beam reflected from thin films, has been applied to the thickness or the optical parameters estimation of a thin film adsorbed on a substrate. It is sensitive enough to detect the adsorption of a molecular layer on a solid surface, and the substrate for it is not confined to a metal surface. It has been used for studies of adsorption of biological molecules to a solid surface such as a gold surface or a silicon wafer [4]. An imaging version of ellipsometry, imaging ellipsometry (IE), allows for the visualization of lateral inhomogeneities in the refractive index of thin films, and has been applied to imaging patterns of Langmuir-Blodgett films and oxide films on a solid surface [10]. IE also has a possibility of the simultaneous detection of several protein bindings as well as the inherent advantages of ellipsometry. It has been reported that IE could be applied to the detection of antigen or microorganism based on an immunoreaction [2, 6]. However, it has not been applied to the detection of *Escherichia coli* O157:H7 (*E. coli* O157:H7).

In this study, IE was applied to the detection of binding of *E. coli* O157:H7 onto the antibody spot immobilized on

*Corresponding author
Phone: 82-2-705-8480; Fax: 82-2-3273-0331;
E-mail: jwchoi@sogang.ac.kr

a solid surface. Antibody was immobilized on the protein G spot formed on a gold surface modified with a self-assembled monolayer (SAM). The immobilization of antibody was investigated using SPR. Ellipsometric images of the Mab spots corresponding to binding of *E. coli* O157:H7 with various concentrations were acquired. Lastly, the possibility of application of IE to an immunosensor for detection of *E. coli* O157:H7 is discussed.

MATERIALS AND METHODS

Materials

Protein G (MW'22,600 daltons) and monoclonal antibody against *E. coli* O157:H7 (Mab) were purchased from Prozyme Inc. (U.S.A.) and Fitzgerald Industries International, Inc. (U.S.A.), respectively. 11-Mercaptoundecanoic acid (11-MUA) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC) were purchased from Sigma Chemical. Other chemicals used in this study were obtained commercially as reagent grade, and the water used in this work was double-distilled water.

Fabrication of Antibody Spot

A silicon wafer, on which chromium (5 nm) and gold (150 nm) layers were deposited in sequence by DC magnetron sputtering, was used as a substrate. A SAM of 11-MUA was formed on the substrate by submerging the substrate into a glycerol/ethanol (1:1, v/v) solution containing 150 mM 11-MUA for at least 12 h. After the substrate had been treated with EDAC, 0.1 mg/ml protein G solution with 10% glycerol was spotted on the substrate using the inkjet-type micro-arrayer (Nano-Plotter Model 1.2, GeSiM mbH, Germany), followed by incubation of the substrate during at least 24 h. After the substrate was blocked with 3 w% bovine serum albumin (BSA), 10 μg/ml Mab was applied to the substrate.

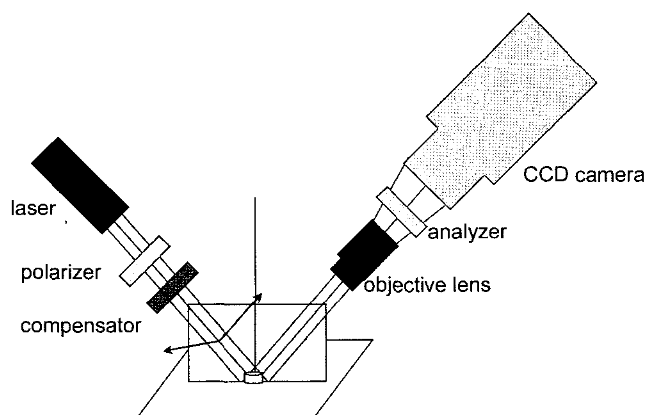


Fig. 1. Configuration of imaging ellipsometry system based on off-null ellipsometry.

Imaging Ellipsometric Analysis

Ellipsometric images were acquired using the IE system (Multiskop, Optrel Gbr, Germany) modified from a polarizer-compensator-sample-analyzer (PCSA) ellipsometry system, as shown in Fig. 1 [5]. In the configuration of the IE system, an objective lens (×10) was placed in between the sample and the analyzer to magnify the intensity profile of the cross-section of the beam reflected from the sample, and the CCD camera was used to capture the magnified intensity profile as a two-dimensional image with resolution of 640×480 pixels. In all experiments using the IE system, the incident angle of the laser beam (632.8 nm) was set to 40°.

The SPR curves were measured using the Kretschmann configuration modified from the null-ellipsometry system.

RESULTS AND DISCUSSION

SPR Analysis

The SPR curves corresponding to the depositions of 11-MUA, protein G, Mab layer, and 10^5 CFU/ml *E. coli* O157:H7 on a gold surface are shown in Fig. 2. BK7 glass (18 mm×18 mm, Superior, Germany), on which chromium (5 nm) and gold (43 nm) were deposited in sequence, was used as a substrate. The SPR angle of the substrate was 43.016°±0.035. In principle, the SPR angle, at which the reflectivity was minimized, is positively shifted with adsorption of dielectric layer on a metal surface. The positive shifts of the SPR angle of the substrate with depositions of each layer were observed in the SPR curves. The SPR angle corresponding to the adsorption of the SAM of 11-MUA was shifted to 43.214°±0.030. A SAM is formed with a close packed configuration by means of the

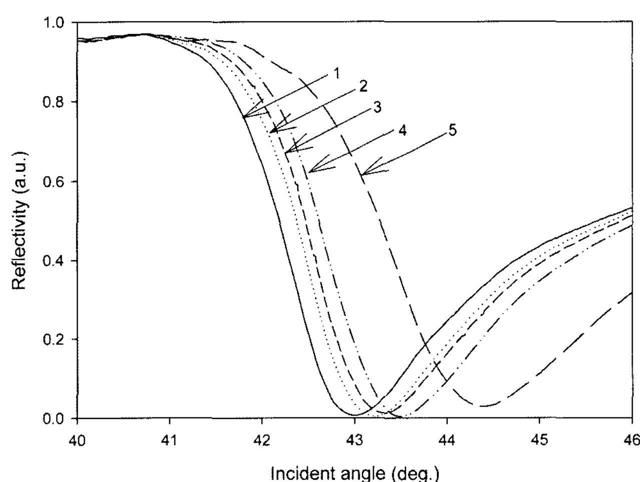


Fig. 2. SPR curves of a bare gold surface (1) and gold surfaces with deposition of 11-MUA (2), protein G (3), Mab (4), and *E. coli* O157:H7 (10^5 CFU/ml) (5), respectively.

van der Waal's attractive force among the long alkyl chains, and the surface property of substrate can be controlled via changing a terminal of molecule. The 11-MUA layer with a carboxyl group as the terminal group was used in order to chemically bind protein G to it. Chemical binding of the protein G layer to the SAM treated with EDAC shifted the SPR angle to 43.327°±0.041. The protein G used in this study was a recombinant form of streptococcal protein G, with two binding domains to an Fc region of antibody and a lysine-rich domain. As the Mab was immobilized on the protein G layer, the SPR angle was shifted to 43.469°±0.065. Because an antibody is immobilized on the protein G layer with a configuration in which the Fab domains face away from the substrate, the binding capacity of antibody to antigen can be improved [8, 9]. Lastly, the SPR angle was shifted to 44.044°±0.076 because of the binding of 10^5 CFU/ml *E. coli* O157:H7 onto the Mab layer. The SPR experiment indicated that the immobilization protocol was suitable for immobilization of Mab on a substrate.

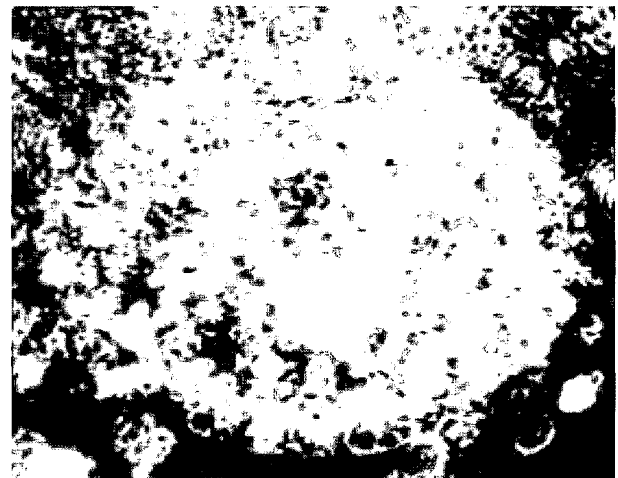
Ellipsometric Image of Protein Spots

Fig. 3A shows the ellipsometric image of the protein G spot blocked with BSA. Because the azimuths of the polarizer and analyzer were set respectively to 126.0°±0.3 and 136.0°±0.3 in order for the intensity of beam reflected from the BSA region to be extinguished, the darkest region surrounding the protein spots was the region blocked with BSA. Although, in a preliminary experiment with FITC-labeled protein G, it was observed that the protein spots formed using the micro-arrayer were a circular shape of diameter about 120 μm, they looked oval shaped in the ellipsometric

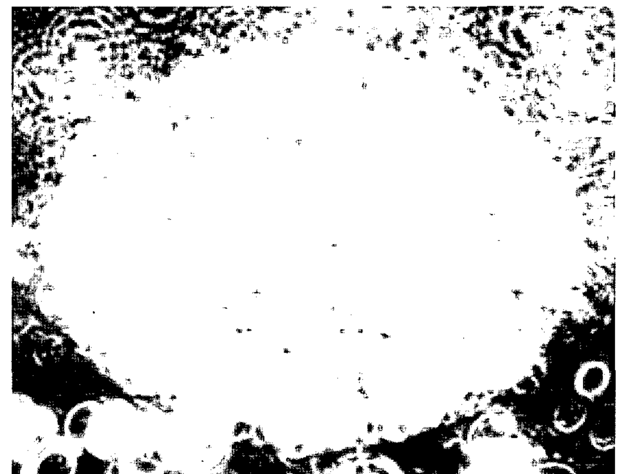
A

B

Fig. 3. Ellipsometric images of the protein G spot blocked with BSA (**A**), and the Mab spot with Mab immobilized on the protein G spot (**B**).



A



B

Fig. 4. Ellipsometric images of the Mab spots to which *E. coli* O157:H7 was bound in concentrations of 10^4 CFU/ml (**A**) and 10^7 CFU/ml (**B**).

image. The reason for this was that the laser beam was irradiated into the sample at an oblique angle (40°). The mean optical intensity (MOI) of the protein G spot region was 241.1±1.7, which was nearly the saturated value, considering that the optical intensity value ranged from 0 to 255 (8 bits signal) in the CCD camera. The IE system is based on off-null ellipsometry, in which an optical intensity of the beam reflected from a sample is proportional to the square of the thickness of the thin film on a solid film [1]. Thus, the local difference of the optical intensity in the magnified cross-section of the reflected beam indicated that the thickness of the protein layer was locally inhomogeneous in the area irradiated; that is, the thickness of the protein G spot was deviated from that of the BSA region in the ellipsometric image.

The Mab spot was completed via immobilizing Mab on the protein G spot. Fig. 3B shows the ellipsometric image of the Mab spot. The MOI of the Mab spot was 185.7±4.1, which was lower than that of the protein G spot. According to the immobilization of Mab, the thickness difference between the Mab spot and the BSA region became lessened, which resulted in the decrease of the MOI of the Mab spot.

Detection of *E. coli* O157:H7

After *E. coli* O157:H7 in various concentrations had bound to the Mab spot, their ellipsometric images were acquired. The ellipsometric images of the Mab spot with 10^4 CFU/ml and 10^7 CFU/ml *E. coli* O157:H7 are shown in Fig. 4. It was observed that as the concentration of *E. coli* O157:H7 increased, the local white areas in the Mab spot increased. Considering that the general size of a microorganism is about 0.5–5 μm, local deviations of thickness in the Mab spot due to binding of the microorganism to the Mab spots can be generated. Such local deviations of thickness

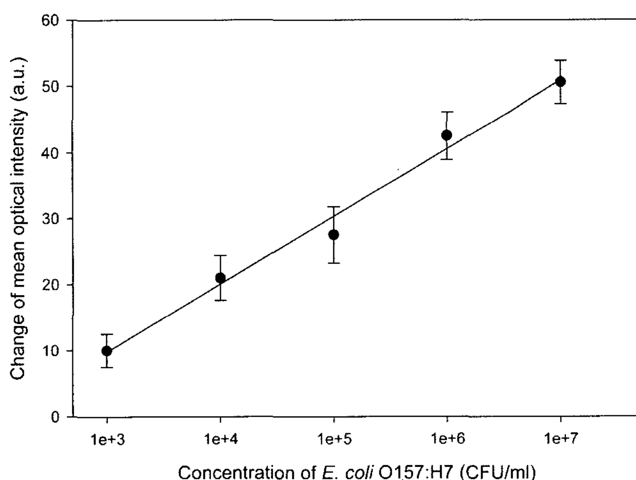


Fig. 5. Changes of the MOI of Mab spot as a function of *E. coli* O157:H7 concentration.

resulted in the local changes of optical intensity in the Mab spot.

Fig. 5 shows the relative changes of the MOI of the Mab spots to the MOI of the Mab spot without the binding of *E. coli* O157:H7 as a function of *E. coli* O157:H7. Significant change of MOI appeared beginning with 10^3 CFU/ml *E. coli* O157:H7, and it was proportional to the logarithmic value of concentration. The MOI of the Mab spot with binding of 10^7 CFU/ml of *E. coli* O157:H7 was 236 (a.u.)±3.2. The result was superior to a standard enzyme-linked immunosorbent assay (ELISA), of which the detection limit is known to be about 10^6 CFU/ml [7]. Conclusively, IE can be applied to the development of an immunosensor for detection of *E. coli* O157:H7 with high sensitivity, adding to the advantage of allowing for label-free detection.

Acknowledgments

This work was supported by the Korea Science and Engineering Foundation (KOSEF) through the Advanced Environment Monitoring Research Center at Gwangju Institute of Science and Technology and by the Nano/Bio Science & Technology Program (M10536090001-05N3609-00110) of the Ministry of Science and Technology (MOST), and by the Brain Korea 21 project of the Ministry of Education and Human Resources Development.

REFERENCES

1. Arwin, H., S. Welin-Klintström, and R. Jansson. 1993. Off-null ellipsometry revisited: Basic considerations for measuring surface concentrations at solid/liquid interfaces. *J. Colloid Interface Sci.* **156:** 377–382.
2. Bae, Y. M., B.-K. Oh, W. Lee, W. H. Lee, and J.-W. Choi. 2003. Immunosensor for detection of *Legionella pneumophila* based on imaging ellipsometry. *Mat. Sci. Eng. C* **24:** 61–64.
3. Brecht, A. and G. Gauglitz. 1995. Optical probes and transducers. *Biosens. Bioelectron.* **10:** 923–936.
4. De Feijter, J. A., J. Benjamins, and F. A. Veer. 1978. Ellipsometry as a tool to study the adsorption behavior of synthetic and biopolymers at the air-water interface. *Biopolymers* **17:** 1759–1772.
5. Harke, M., R. Teppner, O. Schulz, H. Motschmann, and H. Orendi. 1997. Description of a single modular optical setup for ellipsometry, surface plasmons, waveguide modes, and their corresponding imaging techniques including Brewster angle microscopy. *Rev. Sci. Instrum.* **68:** 3130–3134.
6. Jin, G., P. Tengvall, I. Lundström, and H. Arwin. 1995. A biosensor concept based on imaging ellipsometry for visualization of biomolecular interactions. *Anal. Biochem.* **232:** 69–72.
7. Oh, B.-K., Y.-K. Kim, Y. M. Bae, W.-H. Lee, and J.-W. Choi. 2002. Detection of *Escherichia coli* O157:H7 using

immunosensor based on surface plasmon resonance. *J. Microbiol. Biotechnol.* **12:** 780–786.

8. Oh, B.-K., W. Lee, Y. M. Bae, W. H. Lee, and J.-W. Choi. 2003. Surface plasmon resonance immunosensor for detection of *Legionella pneumophila*. *Biotechnol. Bioprocess. Eng.* **8:** 112–116.
9. Oh, B.-K., W. Lee, W. H. Lee, and J.-W. Choi. 2003. Nano-scale probe fabrication using self-assembly technique and application to detection of *Escherichia coli* O157:H7. *Biotechnol. Bioprocess. Eng.* **8:** 227–232.
10. Rotermund, H. H., G. Haas, R. U. Franz, R. M. Tromp, and G. Ertl. 1995. Imaging pattern formation in surface reactions from ultrahigh vacuum up to atmosphere pressures. *Science* **270:** 608–610.
11. Stelzle, M., G. Weissmüller, and E. Sackmann. 1993. On the application of supported bilayers as receptive layers for biosensors with electrical detection. *J. Phys. Chem.* **97:** 2974–2981.