이미지 엘립소미트리를 이용한 예시니아 검출용 바이오센서 개발

Biosensor for Detection of *Yersinia enterocolitica* based on imaging ellipsometry

배영민* 박광원** 최정우** 조성인***
정회원 정회원
Y. M. Bae K. W. Park J.-W. Choi S. I. Cho

1. Introduction

The immunosensor based on antigen-antibody binding have been developed for detecting several analytes including antigen, small molecules, and cell. This method can be rapid and show very good detection limits. For implementation of immunosensor, technologies for immobilization of antibody onto solid surface and detection of protein-protein binding must be developed.

Yersinia enterocolitica is one of the human pathogenic species of *Yersinia* genus and has emerged as a significant foodborne pathogen. Human infections due to *Y. enterocolitica* as a cause of pediatric entercolitis known as yersiniosis that is characterized by fever, diarrhea, and abdominal pain have increased dramatically during the past two decades. A number of the techniques based on polymerase chain reaction (PCR) assay were developed for detection of *Y. enteroclitica*. Although the PCR based method has the potential of detection of pathogen with high sensitivity, this method needs relatively expensive equipment and associated specialist skills to perform the analyses. Therefore, it is meaningful to develop an alternative method to detect *Y. enterocolitica* with high sensitivity, with a short detection time and with simplicity.

In this study, an immunosensor based on the IE as a novel detection method (Jin,et al., 1996) is reported for detection of *Y. enterocolitica*. For immobilization of antibody, a binding protein layer, which is chemically adsorbed onto gold surface, is adopted. Deposition of each layer onto gold surface in sequence was investigated using the SPR, and the immunosensor is fabricated with a micro-arrayer. Lastly, the IE is applied to detect binding of *Y. enterocolitica* onto the surface of the immunosensor.

* 서강대학교 산업기술연구소
** 서강대학교 화학공학과
*** 서울대학교 생물자원공학부

2. Materials and Methods

2.1 Materials

Protein G (M.W. 22600 daltons) was purchased from Prozyme Inc. (USA) which is a recombinant protein G capable of binding the Fc portion of Ig.G. Monoclonal antibody against E. coli O157:H7 was obtained from Fitzgerald Industries International, Inc. (USA). Other chemicals used in this study were obtained commercially as the reagent grade.

2.2 Immobilization of antibody

A thin layer of 11-mercaptoundecanoic acid (11-MUA) on the gold surface was prepared by submerging the substrate into a glycerol/ethanol (1:1, v/v) solution containing 150mM 11-(MUA) for at least 12 hours. For chemical binding between11-MUA adsorbed on Au and free amine of protein G, the carboxyl group of 11-MUA was activated by submerging the substrate modified with 11-(MUA) into a solution of 10% 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC) in water/ethanol (10/1, v/v) for 2 hours at room temperature.

The protein G solution was spotted on the activated surface with the inkjet-type micro-arrayer (Nano-Plotter Model 1.2, GeSiM mbH, Germany). The spotted amount per each spot was 0.4nl of a solution of 7.0㎎/㎖ protein G in the mixed solution of 10mM phosphate buffer saline (PBS, pH 7.4) buffer, and 10 vol.% glycerol. The spotted chips were incubated in humid chamber at 4℃ for at least 24 hours. After the incubation, the chip was washed with PBS buffer for 20 to 30mins.

Before the immobilization of the antibody, the residue carboxyl groups of 11-(MUA) on the chip were inactivated by blocking it with 3 w% bovine serum albumin (BSA). A solution containing the antibodies (10㎍/㎖ Mab against E. coli O157:H7) in PBS buffer was applied to the blocked chip. After incubated in 4℃ during 3 hours, the surface was washed with PBS buffer with 0.1% Tween 20.

2.3 Imaging ellipsometry

Imaging ellipsometry configuration, based on off-null ellipsometry, which has a component sequence of a polarizer-compensator-sample-analyzer (PCSA) as shown in Fig. 1 (MultiskopTM, Optrel Gbr, Germany), was used (Rotermund, et al., 1995; Arwin, et al., 1993). For acquisition of ellipsometric images, objective lens (×10), which plays a role for magnifying the profile of the cross-section of reflected beam against sample, is inserted in between the sample and the analyzer. After the reflected beam passes

through the objective and analyzer, the intensity profile of cross-section of it was recorded as image of 640x480 pixels by means of the CCD camera. The light source is a He-Ne laser beam (632.8nm). The incident angle of the laser beam, was set to 40°. Mean optical intensity values of ellipsometric images of protein spots were calculated with the image processing software (Image-Pro Ver. 4.7, Media Cybernetics, USA).

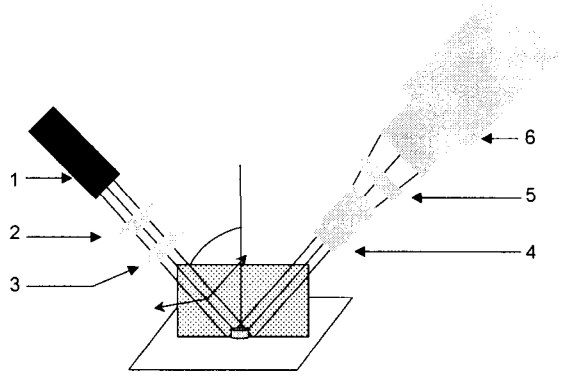


Fig. 1. Imaging ellipsometry system based on PCSA type Null-ellipsometry. 1. polarizer, 2. compensator, 3. objective lens, 4. analyzer, 5. CCD camera.

3. Results and Discussion

3.1 Immobilization of antibody

Before the fabrication of the immunosensor for detection of *Y. enterocolitica*, the deposition of each layer of organic/bio layer on gold was investigated using the SPR. Fig. 2 shows the SPR curves according to the deposition of 11-MUA, protein G, and the antibody layer on the gold surface. The incident angle at minimum reflectance is called as the surface plasmon resonance angle (SPR angle) in the SPR curve, and it is positively moved as thickness and refractive index of the dielectric layer deposited on gold surface increase. The SPR angle about the bare gold surface is 43.02 °±0.07. According to deposition of 11-MUA layer onto gold surface via self-assembly of it, the SPR angle was shifted to 43.24 °±0.06. The amount of shift of the SPR angle was 0.22. After protein G solution was applied to the modified surface, the SPR angle of the substrate was shifted to 43.46 °±0.06. It is known that protein G has two sites that can bind the Fc site of antibody, and an antibody layer has the immobilized on protein G layer with well-orientated configuration. In such a well-oriented configuration of antibody layer, usually, because the Fab site which can bind with specific antigen is faced forward the side opposite to surface, the sensitivity of immunosensor can be improved.

Lastly, the antibody solution against *Y. enterocolitica* (Mab) was applied to the surface with protein G layer. After incubated for 2 hours at room temperature, the

substrate was washed with PBS buffer and water. According to immobilization of antibody layer, the SPR angle was shifted to 43.71 °±0.08. Such a shift of the SPR angle means that antibody was immobilized on the substrate.

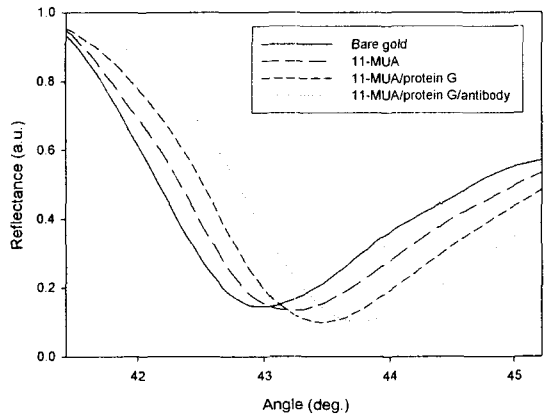


Fig. 2. Surface plasmon resonance curves with deposition of 11-MUA, protein G, the Mab.

3.2 Ellipsometric images of protein spot

After incubation of the spotted chip, in order to minimize non-specific binding of proteins, parts except protein G spot on the surface were inactivated via blocking with BSA. After applying of the solution of the Mab to the chip, incubation, and washing with PBS buffer in sequence, the immunosensor was completed. Fig. 3(a) shows the ellipsometric image of the protein G spot with BSA-blocking. At a glance, the protein patterns were closer to elliptical shape than circle. Such distortion of protein patterns was due to the oblique angle of incidence.

Fig. 3(b) shows the ellipsometric image of the antibody spot immobilized on the protein G spot. It can be easily identified that the image of antibody spot was darker than that of protein G spot. Optical intensities were 243.8±0.2, (a.u.) and 177.0±4.3 (a.u.) for the protein G spot, and the protein spot with immobilization of the Mab, respectively. It indicates that the refractive index of protein spots increased with immobilization of the Mab.

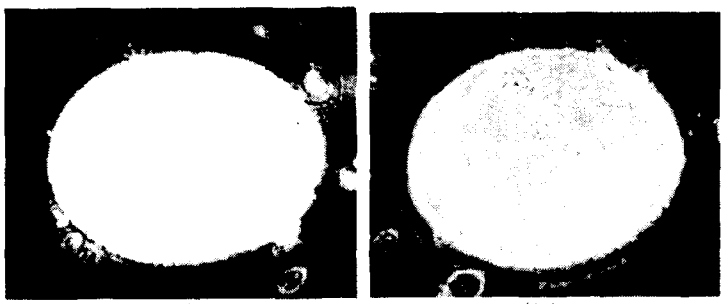
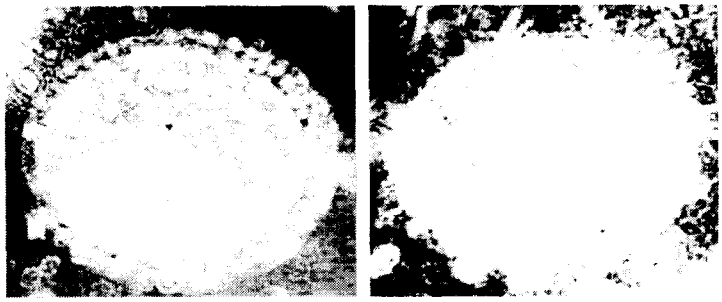


Fig. 3. Ellipsometric images of (a) protein G spot and (b) Mab spot on protein G.

3.3 Detection of *Y. enterocolitica*

The pathogenic solutions with several concentrations were applied to the completed immunosensor chips. After incubation and washing of them with PBS buffer, they were dried at room temperature. Typical ellipsometric images of protein spots, on which the binding of the pathogen to the Mab spot was completed, are shown in Fig. 4. It was observed that as the concentration of the pathogen increased, the white regions in the protein spot increased, lastly, the region of the protein spot became totally white. *Y. enterocolitica* is with rod-shape, and the general size of bacteria is 0.5~5㎛. Thus, since the region to which the pathogen was bound to the chip become thicker than the BSA layer, it was considered that the intensity in the region increased. In Fig. 4(a), the ellipsometric image on which 10^5 CFU/ml solution of the pathogen was applied, the diameter of white regions in protein spot was estimated to be about 7㎛, which is more than the size of bacteria. The reason for it could be due to the aggregation of the pathogen.



(a) (b)

Fig. 4. Ellipsometric images with binding of (a) 10^5 CFU/ml and (b) 10^7 CFU/ml pathogen onto immunosensor.

The mean optical intensities (MOI) of each protein spot were calculated. The change of the MOIs against the concentration of the pathogen is shown in Fig. 5. The outbreak of the MOI appeared at the protein spot with binding of 10^3 CFU/ml cell, and it linearly increased to 10^7 CFU/ml, proportional to the logarithm of concentration of the pathogen. The MOI of the protein spot with reaction with 10^7 CFU/ml was 232.7±2.1(a.u.), which was almost saturated, because output signal from 8 bit CCD camera ranges from 0 to 255. However, large standard deviation was observed. The reason for it was that residue salts from PBS buffer affected the ellipsometric image. In the low concentration, the signal noise due to the residue salts might result in enhancement of the error of the immunosensor based on the IE. For solution for the problem, it will be valuable to operate the immunosensor on in situ flow system. In view of detection limit, it was considered that the detection of pathogen using the immunosensor chip based on the IE was comparable with the other methods.

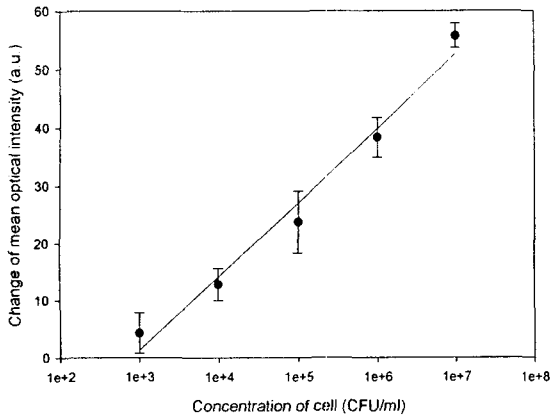


Fig. 5. Change of optical intensity of protein spots according to several concentration of the pathogen.

Acknowledgement

This work was supported in part by the Korea Science and Engineering Foundation (KOSEF) through the ADvanced Environmental Monitoring Research Center at Kwangju Institute of Science and Technology.

4. Reference

1) Arwin, H., S. Welin-Klintstrom, and R. Jansson. 1993. Off-null ellipsomery revisited: Basic considerations for measuring surface concentrations at solid/liquid interfaces. Journal of Colloid and interface science 156: 377-382.

2) Jin G., P. Tengvall, I. Lundstrom, and H. Arwin. 1996. A biosensor concept based on imaging ellipsometry for visualization of biomolecular interactions. Analytical Biochemistry 232: 69-72.

3) Rotermund, H. H., G. Haas, R. U. Franz, R. M. Tromp, and G. Ertl. 1995. Imaging pattern formation in surface reactions from ultrahigh vaccum up to atmosphere pressures. Science 270:608-610.