Biotechnology and Bioprocess Engineering **2004**, 9: 118-126

Application of an Interferometric Biosensor Chip to Biomonitoring an Endocrine Disruptor

Sung-Hyuk Lim and Byung-Woo Kim*

Department of Chemical Engineering, Sungkyunkwan University, Suwon 440-746, Korea

Abstract Recombinant *E.coli* ACV 1003 (*recA::lacZ*) releasing β-galactosidase by a SOS regulon system, when exposed to DNA-damaging compounds, have been used to effectively monitor endocrine disruptors. Low enzyme activity of less than 10 units/mL, corresponding to a μg/L (ppb) range of an endocrine disruptor (tributyl tin, bisphenol A, *etc.*), can be rapidly determined, not by a conventional time-consuming and tedious enzyme assay, but by an alternative interferometric biosensor. Heavily boron-doped porous silicon for application as an interferometer, was fabricated by etching to form a Fabry-Perot fringe pattern, which caused a change in the refractive index of the medium including β-galactosidase. In order to enhance the immobilization of the porous silicon surface, a calyx crown derivative (ProLinker A) was applied, instead of a conventional biomolecular affinity method using biotin. This resulted in a denser linked formation. The change in the effective optical thickness versus β-galactosidase activity, showed a linear increase up to a concentration of 150 unit β-galactosidase/mL, unlike the sigmoidal increase pattern observed with the biotin.

Keywords: interferometeric biosensor, porous silicon, biochip, endocrine disruptor, recombinant *E. coli*

INTRODUCTION

A number of chemicals in the environment have been shown to mimic the actions of steroid hormones. This issue is often described as endocrine disruption/modulation. High incidences of inter-sexuality and reproductive failure, in wild populations of river and marine fish, oysters and gastropods, have frequently been reported [1-3]. These reproductive disturbances are consistent with exposure to hormonally active substances and associated with discharges from sewage treatment works containing estrogenic chemicals. Although a number of chemical tests exist, these tests are quite complex and time-consuming. Moreover, they only indicate how much of a component exists in a sample, not how much is available to living cells. The latter is important for risk assessment and environmental quality analysis. In water and wastewater treatment, unknown chemicals toxic to biota are difficult to measure because the water contains many unknown components. A method to detect potentially damaging toxic levels of multiple pollutants in surface and ground water is required, especially for the development of an early warning system.

A sensor consists of a chemically selective layer, a transducer and a signal processor. If the selective layer utilizes a biological or biochemical species, it can be classified as a biosensor [4]. A biological transducer is comprised of a receptor and the regulatory element (operator/promoter sequences) that control expression of the biodegradative operon, cloned upstream from reporter functions. Several reporter systems, such as luciferase and β-galactosidase, are widely used in studies of transcription regulation. In *E. coli*, DNA damage coordinately induces a set of genes, resulting in several physiological changes, termed the SOS response [5]. Bacterial repair of DNA damage is mediated by at least two inducible systems: the *recA*-independent, *ada*-controlled adaptive response and the *recA*-dependent, *lexA*-controlled response [6]. The SOS response in the latter case, which occurs in the recombinant *E. coli* ACV 1003 adopted in this study, is induced by a cascade mechanism. Following DNA damage, the *recA* protein is activated by damaged DNA [7]. Activated *recA* protein cleaves the *lexA* repressor resulting in the derepression of several genes. By utilizing this cascade, DNA damage transcriptionally induces SOS genes. The products of many of these genes are known to repair DNA. Following DNA damage, these *SOS::lacZ* fusion strains produce β-galactosidase. Recombinant *E. coli* applied in this study releases β-galactosidase when exposed to endocrine disruptors.

Many of the gene products, however, are difficult to assay because of the nature of their enzymatic activities and the particular substrates upon which they react. Unlike a conventional enzyme assay, an inexpensive and easily available, optically-flat, thin film of porous silicon has been used for the highly sensitive detection of short

***Corresponding author**
Tel: +82-31-290-7246 Fax: +82-31-290-7272
e-mail: bwkim@skku.ac.kr

oligonucleotides and proteins. One of the most common commercial biosensors is a surface plasmon resonance method like the BIAcore product with an affinity sensor of IAsys, which uses a waveguide technique, as well as a microfluidic cell. This product has a critical problem in the background signal due to thermal difference, mechanical change and bulk index effects, while labeling with radioactivity or fluorescence is not needed [8,9].

An alternative interferometric method, not using the waveguide and microfluidic cell, has been used in this study in order to solve the problems of the surface plasmon resonance method. It also solves the problems of a conventional enzyme assay, such as, time-consuming procedures in experiment, the use of costly biochemicals and tedious cell collection. This method is based on estimating the change in the effective optical thickness of a thin film of porous silicon due to adsorption of a complete protein monolayer on the functionalized porous silicon surface.

Several protein immobilization methods, for the functional biochip in the surface plasmon resonance or in the interferometric method, have been applied, such as, non-covalent adsorption [10,11], covalent linkage by chemical bonding [12-15] and affinity capture methods like streptavidine-biotin interaction [16,17]. The non-covalent method has problems with the control of orientation or quantitative adsorption of biomolecules, which results in poor reproducibility and lower interaction efficiency. Covalent bonding causes loss of activity and low immobilization efficiency, which requires multiple functional surfaces. However, multiple functional surfaces tend to cause non-uniform noise in the refractive index, therefore, they could not be adopted in this study.

Biomolecular affinity interaction, which causes changes in specificity and affinity of the capture antibody has been widely used. Janshoff *et al.* [17] demonstrated that 10 μM streptavidin was detected as an increase in the effective thickness, due to the adsorption of the protein to the biotin receptors immobilized on the porous silicon surface. Lin *et al.* [18] showed that complementary DNA from 2×10^{-6} to 2×10^{-15} M could be quantified by this method. An alternative immobilization method using a calyx crown derivative (ProLinker A) [19] has been used to obtain a higher immobilization efficiency without activity loss or incorrect orientation for the capture protein, a phenomena which used to occur in the affinity interaction.

MATERIALS AND METHODS

Strain, Growth of Cells and β-galactosidase Assays

The bacterial strain used in this study is ACV 1003, which was transformed by plasmids pRecALac1 and pUvrALac2 into the isogenic strain RFM443 [20]. Included among other genes expressing the SOS response are, *umuC* and *umuD* (induced mutagenesis), *sulA* (filamentous growth), *uvrA* and *uvrB* (excision repair), *himA* (site-specific recombination) and several *din* (damage-inducible). All these genes are members of a regulatory network controlled by the products of the *recA* and *lexA* loci, which are inducible for themselves. Other strains such as GW1010, 1030, and 1040 based on GW1000 [21], made by using the Mu *d* (Ap *lac*) bacteriophage to generate *lac* operon fusions to various *din* genes [22] were also used. However, ACV 1003 was the most effective bacterial strain in its response to the endocrine disruptor (Fig. 1).

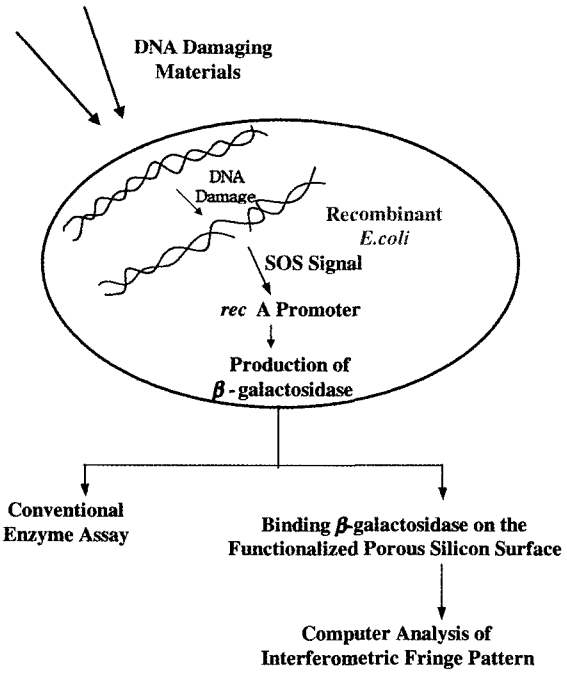

Fig. 1. DNA damaging scheme and analysis.

Luria-Bertani medium for liquid and plate cultures was used. Cells were inoculated into 10 mL of liquid medium in a 250-mL shaking flask, after incubation in the plate culture of Luria-Bertani medium supplemented with 10 μg/mL of ampicillin. Culture proceeded until the optical density reached 0.2, then the tributyl tin was added. Samples were prepared for the conventional β-galactosidase assay as follows [23].

A 0.1 mL chloramphenicol solution (3 mg/L) was mixed with 1 mL samples every 30 min and its optical density measured at 600 nm. After the remaining solution of 0.1 mL chloramphenicol was stored in ice water, it was mixed with 0.9 mL Z-buffer, 0.01 mL SDS (sodium dodecyl sulfate; 0.1%), and 0.01 mL chloroform. This solution, after rigorous shaking, was mixed with 0.2 mL ONPG (*o*-nitropheny-β-D-galactopyranosidase; 4 mg/mL). The reaction time of the enzyme with ONPG was recorded until the reaction was stopped by adding 1 M Na_2CO_3 to this solution, which shifts the pH to 11. Optical densities of the supernatant after centrifugation of this solution were measured at 420 and 550 nm. β-galactosidase activity was estimated as the difference in optical densities at 420 and 550 nm divided by the optical density at 600 nm.

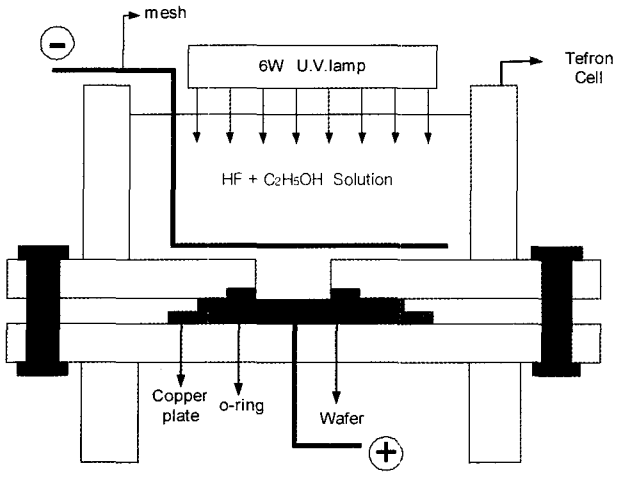

Fig. 2. An anodic etching cell.

Preparation of the Porous Silicon Layer and Measuring the Fabry-Perot Reflectance Fringe

Heavily doped "p"-type silicon wafer [boron-doped and an orientation (100) of the crystal structure] with a resistivity in the range 0.6~1.0 mΩ was anodically etched for several time intervals using HF/EtOH (1:1, v/v). Deposited silicon wafer was annealed by a thermal evaporation method to form ohmic contact in H_2 at 450°C for 30 min [24]. Prior to etching, the silicon wafer was rinsed thoroughly with ethanol, dried under a stream of N_2 and then immersed in the etching solution. The anodic etching of silicon was performed by supplying DC current of 25 mA/cm^2 under illumination by a 60 W UV lamp, with a peak emission at 254 nm, to adjust the thickness of the porous silicon layer (Fig. 2). The interference or fringe pattern was measured to quantify the optical characteristic of porous silicon. The concentration of β-galactosidase was sensed by the change in the Fabry-Perot fringes obtained from the porous silicon layer, which was immersed for 30 min in the enzyme solution and then purged with N_2.

A tungsten light source was installed in a dark container and focused on the center of a porous silicon surface with a spot diameter of approximately 2 mm. The interferometric reflectance spectrum was recorded with a detector. The spectrum was compared with a spectrum obtained from the same porous silicon sample immersed in pure deionized water, as a reference. Using the difference in the spectra, the change in the effective optical thickness was calculated in order to correlate it with the enzyme concentration. The interferometric reflectance spectra were measured by using a spectrometer (S2000, Ocean Optics, Inc., Dunedin, Florida, USA) with a reflectance probe, which was bundled with six illumination fibers with a read fiber at the center of the probe (Fig. 3).

Functionalization of the Porous Silicon Surface

Dancil *et al.* [25] overcame several key biosensor limitations in label-tree methodologies such as, nonspecific binding (NSB), the appearance of a signal due to weak surface binding of interferents which may be present at a much higher concentration than the analyte and signal stability.

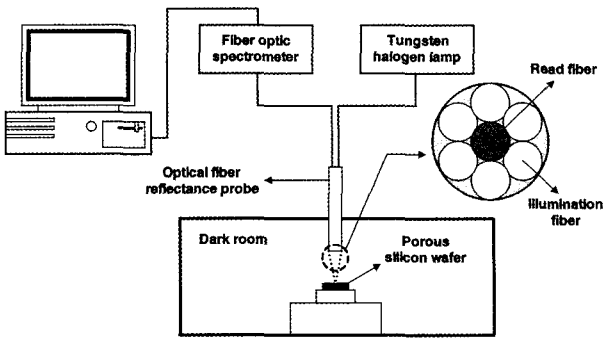

Fig. 3. A computerized interferometer biosensor system.

Three kinds of chemical functionalization of a p-silicon surface are shown in Fig. 4. Major chemical treatment steps in Fig. 4(A) are as follows. The first step of oxidation, for signal stability, is performed by exposing the freshly etched p-Si sample to an ozone environment to reduce signal drift due to oxidation of p-silicon in an aqueous matrix. The second step is to generate a surface-bound pyridyldithio group. The third step is the reaction of the pyridyldithio-modified surface with dithiothreitol (DTT), which results in reduction of the disulfide bond to a SH group. The fourth step is the coupling of the sulfhyhryl-terminal with the succinimide group to modify a carboxyl group to amine reactive NHS (N-hydroxy succinimide) ester. The fifth step is the coupling of the biotinylated HPDP (hexyl-3'-2'-pyridyldithiopropion amide) to the NHS-modified p-Si surface, which is intended to eliminate the nonspecific binding as well as to regenerate the bound protein after cleavage from biotin. In Fig. 4(B), the pyridyldithio group in step 2 in Fig. 4-1, which used to be synthesized with several chemical procedures, was replaced with a commercially available mercaptopropyl-trimethoxyl group for this study. Therefore, the three -NH-C=O- groups in the final functional group were reduced into two groups. In Fig 4(C), ProLinker A was bound with 3-aminopropyltrimethoxysilane directly on the porous silicon surface instead of 3-mercaptopropyltrimethoxysilane with biotin-HPDP.

Instrumental Analysis

SEM (scanning electronic microscopy, JSM6700F, FESEM II, JEOL, Japan) was used to investigate the etched pattern of the silicon surface. XPS (X-ray photoelectron spectroscopy, ESCA 2000, VG Microtech, England) was used for the chemical analysis of (3-mercaptopropyl) trimethoxysilane and (3-aminopropyl) trimethoxysilane layers on the silicon surface. AFM (atomic force microscopy, CP Research, Thermo Microscopes, California, USA) was used to investigate the roughness of the modified surface of the bulk silicon after each step of

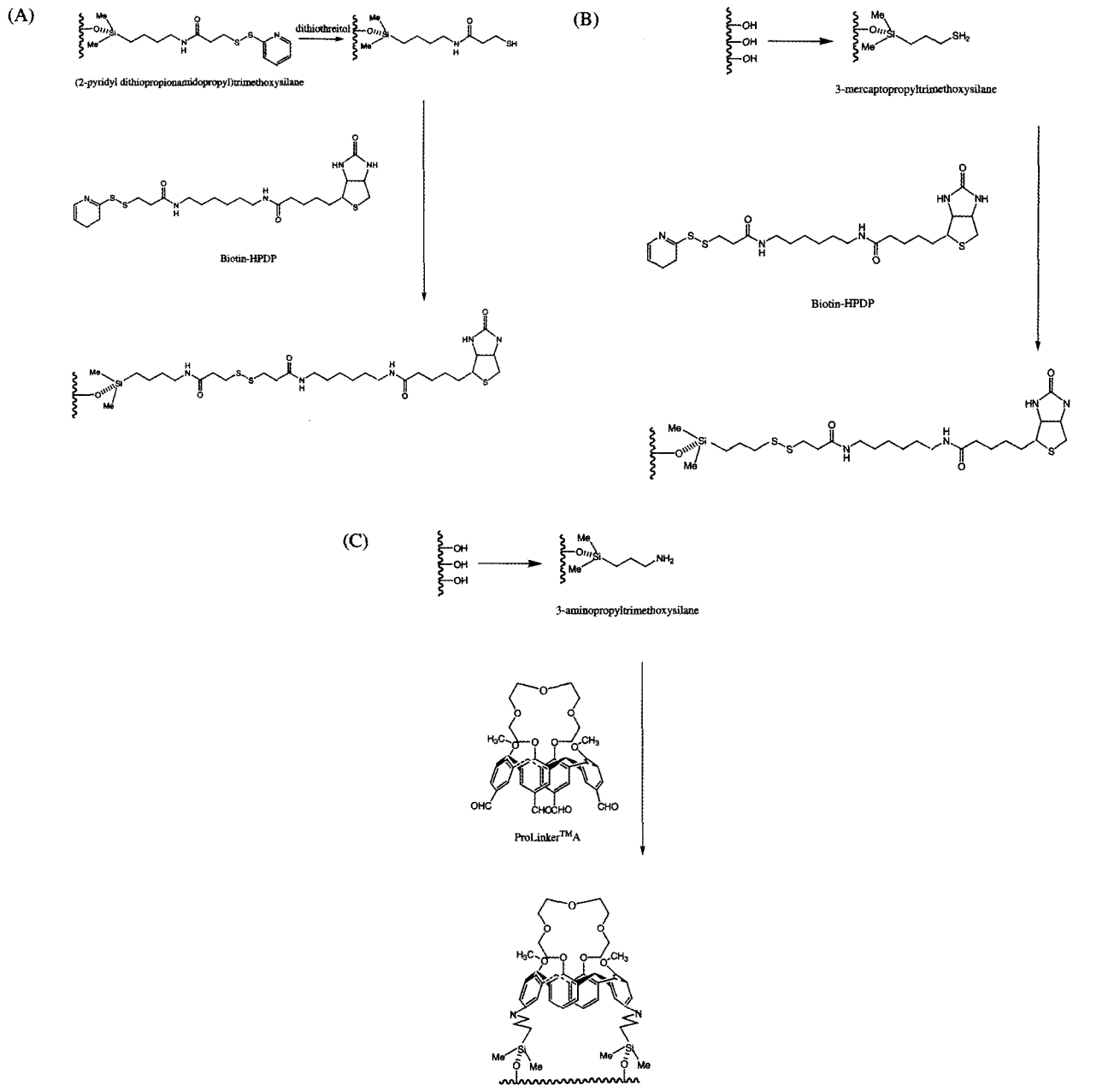

Fig. 4. Functionalization of a porous silicon surface by using biotin with a synthesized pyridyl-dithio group (A), by using biotin with a commercial mercaptopropyltrimethoxyl group (B), and by using Prolinker™A(C)

the functionalization process. In order to confirm the existence of biotin groups in the silicon surface, a FT-IR (Fourier Transform Infrared Spectrometer, FT/IR-660 plus, Jasco, Japan) was used. A UV/Vis spectrophotometer (2120UV, Optizen, Korea) was used in the Miller's conventional enzyme assay.

RESULTS AND DISCUSSION

The *lexA* protein functions as a repressor of all of the *din* genes. Damage to cellular DNA or interference with its replication initiates a series of events that lead to activation of the protease activity of the *recA* protein. The *lexA* protein is then proteolytically cleaved by the *recA* protease, and, as the pools of *lexA* protein decreases the various din genes begin to be expressed at increased levels. Following DNA damage due to exposure to endocrine disruptors, TBT, bisphenol A, *etc.*, the *SOS::lacZ* fusion strain produces β-galactosidase.

Response ratios of applied strains were calculated by dividing the β-galactosidase activity of the treated sample

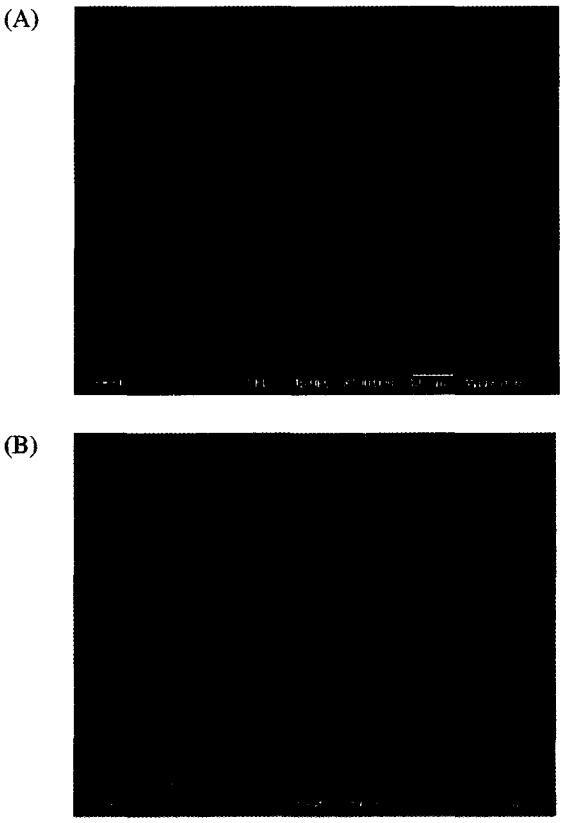

Fig. 5. SEM image of a p-silicon surface before etching (A) and that of an anodically-etched porous p-silicon surface (B)

by the β-galactosidase activity of the untreated sample for the strain ACV1003. The SOS regulon system of ACV1003 was estimated to be more active than the other recombinant *E. coli*, ACV1004, GW1010, GW1030 and GW1040 in a previous study [26].

As explained previously, the assay of β-galactosidase [23] requires several experimental steps that are very time consuming. To analyse β-galactosidase, not by Miller's method, but by an interferometric biosensing method, β-galactosidase activity released from the cell was determined. Extracellular β-galactosidase activity without using extracting solvents was 51 unit/mL, which was 5% of that obtained by the conventional Miller's enzyme assay using solvents. Such a low enzyme unit can be rapidly determined not by the time-consuming and tedious enzyme assay but by an alternative interferometric biosensor.

Determination of an Optimal Etching Condition

Pore structure of a porous silicon surface, is dependent on the etching conditions such as etching time, HF concentration and current density in the dark container. As the etching time increases, the thickness of the pore decreases and then pores disappear by the electropolishing phenomena, while the pore size of the silicon surface increases and then appears flat above a certain size. Investigating the effect of the etching time, on the change of fringe pattern in the reflectance spectrum, led us to the conclusion that the optimal etching time was 120 sec at a current density of 25 mA/cm^2, that resulted in the maximum fringe numbers. The number of fringes decreases and then disappears as the etching time increases over 120 sec. The average pore size of the etched Si surface at 120 sec was about 20~50 nm, which is shown relatively uniformly in Fig. 5.

The Functionalized Biosensor Chip Surface

As explained in Materials and Methods, the porous silicon surface was functionalized with two kinds of linkers, biotin and ProLinker A between a solid Si surface and a target biomolecule-β galactosidase. Before the immobilization of these linkers for the signal stability, the porous Si surface was pretreated by thermal oxidation to reduce signal drift due to oxidation of porous Si in an aqueous matrix, and then functionalized with thio- and amino-groups to reduce the disulfide bond to the SH group. Then the immobilization with biotin and ProLinker A was done by the self assembly method [27].

Fig. 6 shows the AFM (Atomic Force Microscopy) images of the thermally-oxidized Si surface (A), (3-aminopropyl)trimethoxysilane-coupled Si surface (B), ProLinker A-silane coupled Si surface (C), β galactosidase on the ProLinker A-silane coupled Si surface, respectively. Dense formation of the amino-group was shown with 2~3 nm intervals on the porous Si surface in (B). Formation of the ProLinker A group, in (C), was shown to be denser than that of the biotin group, which was not included in this figure. A denser linked formation makes a higher binding capacity with a target biomolecule, which results in an increase of the refractive index. Major refractive indices are summarized in Table 1. White spots in (D) were judged to be β galactosidase with a size of 6 × 8 × 11 nm bound onto the immobilized Si surface [28].

Shift of the Fringe Pattern with β-galactosidase from the Porous Si Layer

Reflection of white light at the top and bottom of the porous Si layer results in an interference pattern relating to the effective optical thickness (product of thickness L and refractive index n) of the film;

$$m\lambda = 2nL$$

where m and λ are the spectral order and the wavelength of the light. Binding of a target biochemical substance to its corresponding ligand or receptor, immobilized on the porous Si substrate, results in an increase in the effective refractive index (ΔnL) of the layer medium and is detected as a corresponding shift in the interference pattern.

Electrochemical etching of Si to produce Fabry-Perot fringes in their optical reflection spectrum generates a thin (1 to 5 μm) layer of porous Si substrate with cavities as wide as 200 nm in diameter, providing a large surface area for biomolecular interaction inside the porous layer

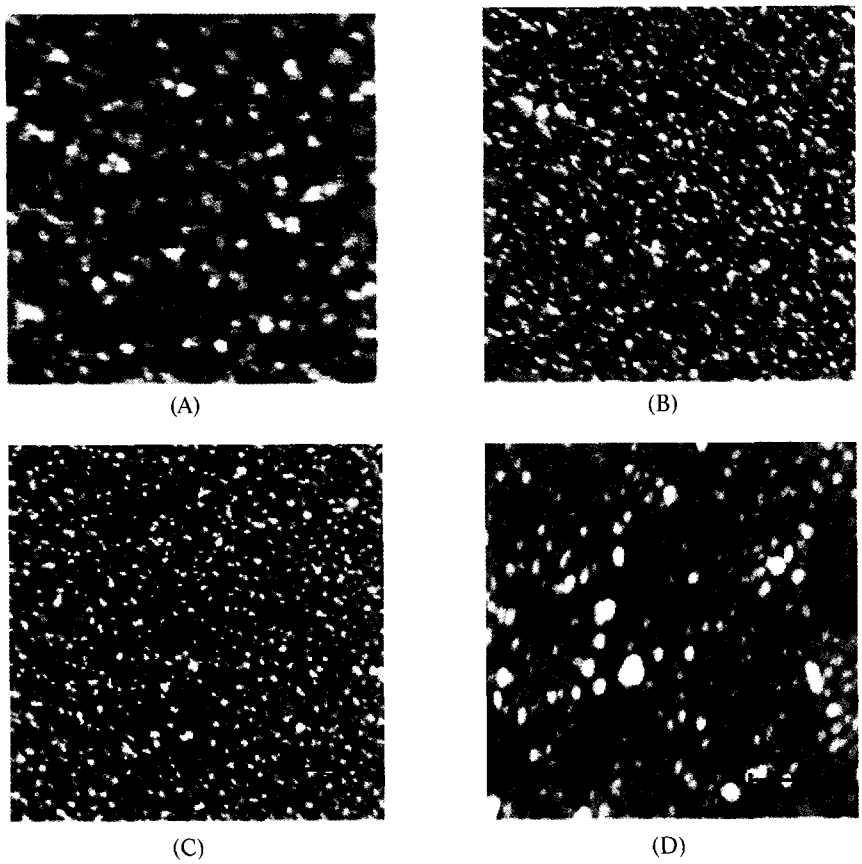

Fig. 6. AFM image of the functionalized silicon surface of 1×1 μm^2 (A: thermal oxidized silicon surface, B: (3-aminopropyl) trimethoxysilane-coupled silicon surface, C: ProLinker^{TM}A-silane coupled silicon surface, and D: β-galactosidase on the prolinker^{TM}A-silane coupled silicon surface, respectively)

[18]. Specific binding of streptavidin to the biotin-coupled porous layer resulted in an increase in a red shift of the fringe pattern. This phenomenon is based on the above equation for constructive interference of two light beams reflected at both interfaces of a thin solid-sup-ported transparent film [17].

The exterior surface area of the porous silicon was 1 cm^2. The changes in fringe patterns were measured from the same porous silicon sample after immersion in β-galactosidase solutions at different concentrations. Those changes were compared with a fringe pattern obtained with pure deionized water on the porous silicon, as a reference. The β-galactosidase was diluted with deionized water in order to obtain various concentrations from 10 to 150 enzyme unit/mL.

In Fig. 7, Fabry-Perot patterns are shown for various concentrations of β-galactosidase. The number of fringes in the observed wavelength range depends on the porosity as well as the thickness of the porous layer. The higher the current density, the fewer fringes can be obtained. This is consistent with the observation that higher current densities lead to samples with greater porosities. Seven fringes were obtained at a DC current of 25 mA/cm^2 in this study as shown in Fig. 7.

The Change of Effective Optical Thickness with β-galactosidase Activity

A change in the refractive index results as a species is adsorbed onto the porous silicon surface, which causes wavelength shifts of the fringe pattern. Exposure of a porous silicon sample to a β-galactosidase containing solution, resulted in a gradual shift to the left side of the interference fringes. A shift in wavelength changed the effective optical thickness, which was estimated as the slope of a peak order m *vs.* $1/\lambda$ curve. Using a general peak-finding algorithm, a straight line with respect to β-galactosidase concentration, was fitted to the graph of m *vs.* $1/\lambda$, not shown in this paper. The slope gave the effective optical thickness.

The change in the effectiveness optical thickness (Δ EOT) was obtained by subtracting the value of EOT with deionized water only, from that of EOT with β-galactosidase. The changes in the effective optical thickness, with the ProLinker A-coupled Si surface, were 41, 78, 103, 177, and 238 nm in 30, 50, 70, 100, and 150 unit β-galactosidase/mL, respectively. While those with the biotin-coupled Si surface were 19, 37, 74, 84, 97, 96, 137, 136, and 147 nm in 10, 20, 30, 50, 60, 70, 100,

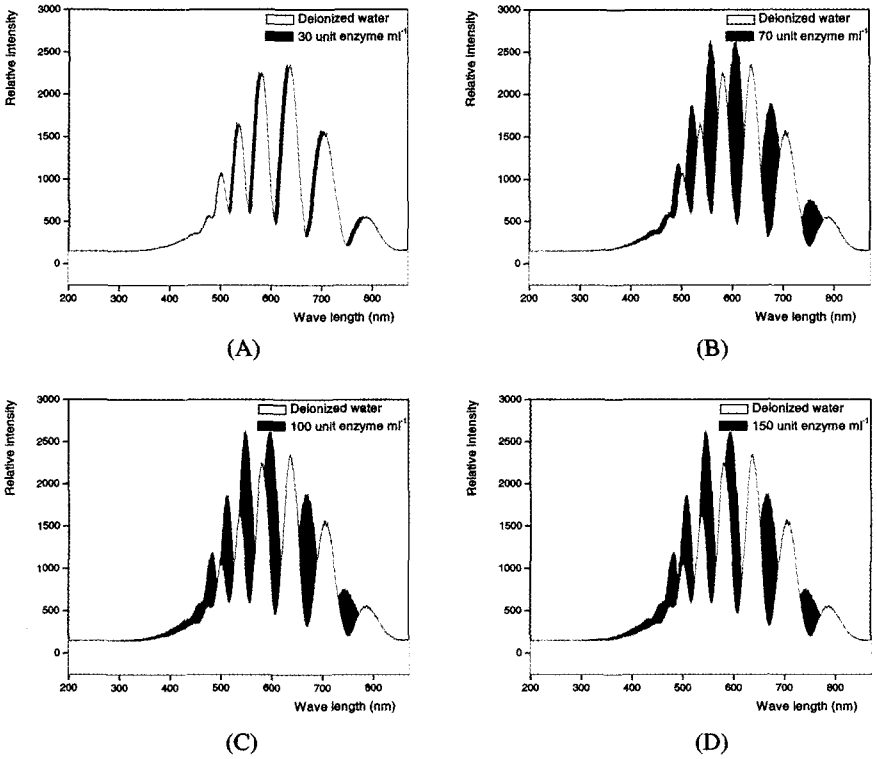

Fig. 7. Interference pattern and differential spectra with a β-galactosidase solution when ProLinker^{TM}A was used as the biological binder (A: 30 units, B: 70 units, C: 100 units, and D: 150 units of β-galactosidase per ml solution, respectively)

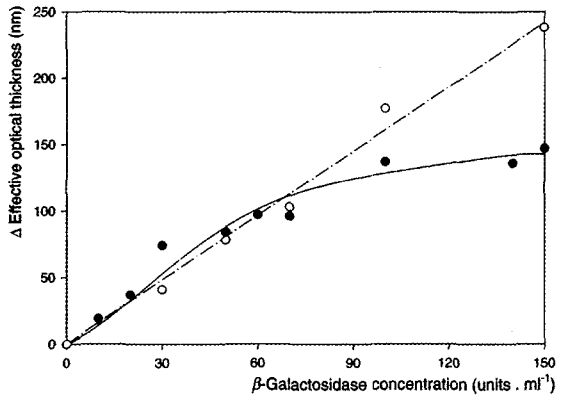

Fig. 8. Effective optical thickness as a function of the β-galactosidase concentration when biotin and ProLinker^{TM}A were used as the biological binders (–: immobilization of β-galactosidase using biotin and – • –: immobilization of β-galactosidase using ProLinker^{TM}A)

140, and 150 unit β-galactosidase/mL, respectively. These changes shown as ΔEOT, are plotted, with respect to β-galactosidase activity, in Fig. 8.

A biotin-coupled porous Si surface can reduce nonspecific binding, while self-assembled alkenethiol and silane monolayers on the porous Si surface have simple functionalities as C- or N-terminals. However, a saturated pattern in the concentration of β-galactosidase vs. ΔEOT is shown above 90 unit β-galactosidase/mL. ΔEOT, in the case of the biotin-immobilized Si surface, increases sharply in low enzyme concentrations and then increases smoothly in about 100 unit β-galactosidase/mL. This results in a sigmoid shaped curve, as in the case of DNA on the porous silicon [18]. As molecules of β-galactosidase occupy the adsorption sites in the porous silicon surface, equilibrium in adsorption proceeds.

However, the self-assemled monolayer of ProLinker shows a linear increase in ΔEOT with enzyme concentrations up to 150 unit/mL. Unlike the biotin-coupled surface, it allows tight binding of β-galactosidase to the crown moiety of the linker molecules without further surface modification (see Fig. 6(C)).

The change in the effective optical thickness (ΔEOT) due to adsorption of a complete protein monolayer with a thickness of 5 nm is 32 nm, using a numerical solution. When 1~10 ng of protein was bound onto 1mm^2 of p-Si surface, 5% of a protein monolayer could be formed [15].

In this study, the thickness of pores in the etched Si surface was estimated to be about 1.5 μm by use of the SEM cross-section image. Since the Δ n=1.42-1.33= 0.09 (Table 1), ΔEOT is about 135 nm when the surface is fully covered with the monolayer of β-galactosidase. During the monolayer formation on the ProLinker bound Si surface, a linear increase of ΔEOT, is advantageous due to the easy determination of enzyme concentration on the ProLinker-A coupled surface.

The interference effect, in a real application of this in

Table 1. List of refractive indices

Material	Refractive Index (n)
Gases at 0°C and 1 atm	
Air	1.000293 [29]
Helium	1.000036 [29]
Hydrogen	1.000132 [29]
Liquids at 20°C	
Benzene	1.501 [29]
Chloroform	1.4459 [15]
Diethylether	1.3526 [15]
Ethanol	1.361 [29]
n-Hexane	1.3749 [15]
PBS buffer	1.33 [30]
Tetrahydrofuran	1.4070 [15]
Water	1.333 [29]
Solids at room temperature	
Fused silica	1.458 [29]
Silicon	3.4 [15]
Streptavidin, Protein	1.42 [30]

terferometric sensing system, can arise from nonspecific interactions of chemical mixtures in the culture media with the adsorption site. Interference from nonspecific adsorption has been tried by binding an antibody anti-β galactosidase onto the porous Si surface. These data will be presented in a further presentation.

CONCLUSION

1) The optimal etching time was 120 sec at a current density of 25 mA/cm^2, which was when the maximum fringe numbers were produced. The number of fringes decreases and then disappears as the etching time increases over 120 sec. The average pore size of the etched Si surface at 120 sec was about 20~50 nm, which was shown to be relatively uniform.

2) Formation of the ProLinker A group on the modified Si surface was shown to be denser than that of the biotin group bound on the porous Si surface. A denser linked formation makes a higher binding capacity with a target biomolecule, which results in an increase of the refractive index.

3) In order to determine a very low β-galactosidase activity rapidly and directly, an interferometric biosensing method was employed. The change in the effective optical thickness caused by a shift in peak wavelength was plotted with respect to β-galactosidase activity. A linear increase up to the activity of 150 unit β-galactosidase/mL, was shown in the case of the ProLinker A-immoblized Si surface, while a sigmoidal increase was shown in the case of the biotin-immobilized Si surface.

In order to inhibit nonspecific adsorption by a mixture in the culture medium of *E. coli* to the silicon matrix, a further study has been undertaken on the binding of anti-β-galactosidase on the ProLinker A-coupled Si surface.

Acknowledgement This work was supported by Ecotechnopia 21 Project of the Korean Ministry of Environment.

REFERENCES

[1] Jobling, S., M. Nolan, C. R. Tyler, G. Brighty, and J. P. Sumpter (1998) Widespread sexual disruption in wild fish. *Environ. Sci. Technol.* 32: 2498-2506.

[2] Briscoe, S. F., C. Diorio, and M. S. DuBow (1995) Luminescent biosensors for the detection of tributyltin and dimethyl sulfoxide and the elucidation of their mechanisms of toxicity, pp. 645-655. In: M. Moo-Young (ed.). *Environmental Biotechnology: Principles and Applications.* Kluwer Academic Pub., The Netherlands.

[3] Bryan, G. W. and P. E. Gibbs (1991) Impact of low concentrations of tributyl tin on marine organisms. In: *Metal Ecotoxicology: Concepts and Applications.* Lewis Pub. Inc., Michigan, USA.

[4] Sadik, O. A., M. M. Ngundi, and F. Yan (2000) Environmental biosensors for organochlorines, cyanobacterial toxins, and endocrine disrupting chemicals. *Biotechnol. Bioprocess Eng.* 5: 407-412.

[5] Walker, G. C. (1984) Mutagenesis and inducible responses to deoxyribonucleic acid damage in *E. coli. Microbiol. Rev.* 48: 60-93.

[6] Vollmer, A. C., S. Belkin, D. R. Smulski, T. K. Van Dyk, and R. A. LaRossa (1997) Detection of DNA damage by use of *E. coli* carrying *recA'::lux, uvrA'::lux,* or *alkA'::lux* reporter plasmids. *Appl. Environ. Microbiol.* 63: 2566-2571.

[7] Heitman, J. and P. Model (1991) SOS induction as an in vivo assay of enzyme-DNA interactions. *Gene* 103: 1-9

[8] Campbell, D. P. and C. J. McCloskey (2002) Interferometric biosensors. In: F. S. Ligler and C. A. Rowe Taitt (eds.). *Optical Biosensors, Present and Future.* Elsevier, Amsterdam, The Netherlands.

[9] Choe, T., I. Park, and S. Hong (2002) Determination of tyrosinase mRNA in melanoma by reverse transcription-PCR and optical mirror resonance biosensor. *Biotechnol. Bioprocess Eng.* 7: 212-215.

[10] Muller, W., H. Ringsdorf, E. Rump, G. Wildburg, X. Zhang, L. Angmaier, W. Knoll, M. Lilet, and J. Spinke (1993) Attempts to mimic docking processes of the immune system. *Science* 262: 1706-1708.

[11] Ziauddin, M. and D. M. Sabatini (2001) Microarrays of cells expressing defined cDNAs. *Nature* 411: 107-110.

[12] Willner, I. and E. Katz (2000) Integration of layered redox proteins and conductive supports for bioelectronic applications. *Angew. Chem. Int. Ed.* 39: 1180-1218.

[13] Kim, E. R. (2002) Applications of self assembled monolayers

[SAMs] for biosensor. *Kor. J. Biotechnol. Bioeng.* 17: 417-427.
[14] Sandres, G. H. S. and A. Manz (2000) Chip-based Microsystems for genomic and proteomic analysis, *Trends in Anal. Chem.* 19: 364-378.
[15] Blawas, A. S. and W. M. Reichert (1998) Protein patterning. *Biomaterials* 19: 595-609.
[16] Koyano, T., M. Saito, Y. Miyamoto, K. Kaifu, and M. Kato (1996) Development of a technique for microimmobilization of proteins on silicon wafers by a streptavidin-biotin reaction. *Biotechnol. Prog.* 12:141-144.
[17] Janshoff, A., K. P. S. Dancil, C. Steinem, D. P. Greiner, V. S.Y. Lin, C. Gurtner, K. Motesharei, M. J. Sailor, and M. R. Ghardiri (1998) Macroporous p-type silicon fabry-fringe layers: Fabrication, characterization, and application in biosensing, *J. Am. Chem. Soc.* 120: 12108-12116.
[18] Lin, V. S. Y., K. Motesharei, K. P. S. Dancil, M. J. Sailor, and M. R. Ghardiri (1997) A porous silicon-based optical interferometric biosensor. *Science* 278: 840-843.
[19] Lee, Y., E. K. Lee, Y. W. Cho, T. Matsui, I. C. Kang, T. S. Kim, and M. H. Han (2003) ProteoChip: A highly sensitive protein microarray prepared by a novel method of protein immobilization for application of protein-protein interaction studies. *Proteomics* 3: 1-16.
[20] Drolet, M., P. Phoenix, R. Menael, E. Masse, L. F. Liu, and R. J. Crouch (1995) Overexpression of RNAase H partially complements the growth defect of an *E. coli* Δ*topA* mutant. *Proc. Natl. Acad. Sci. USA* 92: 3267-3630
[21] Kenyon, C. J. and G. C. Walker (1980) DNA-damaging agents stimulates gene expression at specific loci in *E. coli*. *Proc. Natl. Acad. Sci. USA* 77: 2819-2823.
[22] Krueger, J. H., S. J. Elledge, and G. Walker (1983) Isolation and characterization of Tn5 insertion mutations in the *lexA* gene of *E. coli*. *J. Bacteriol.* 153: 1368-1378
[23] Miller, J. H. (1972) *Experiments in Molecular Genetics*. Cold Spring Harbor Lab., NY, USA.
[24] Dascãlu, D., G. H. Brezeanu, and P. A. Dan (1980) Effect of Si dissolution and recrystalization upon ohmic Al/p-Si contacts. *Appl. Phys. Lett.* 37: 215-217.
[25] Dancil, K. P. S. , D. P. Greiner, and M. J. Sailor (1999) A porous silicon optical biosensor: Detection of reversible binding of IgG to a protein A-modified surface. *J. Am. Chem. Soc.* 121: 7925-7930.
[26] Cha, J. M. and B. W. Kim (2002) Bioassay of environmental endocrine disruptors TBT and DMSO using recombinant *E. coli*. *Bioprocess Biosyst. Eng* 24: 405-410.
[27] Senkevich, J. J., G. R. Yang, and T. M. Lu (2002) Thermal stability of mercaptan terminated self-assembled multilayer films on SiO2 surfaces. *Colloids and Surfaces A: Physicochem. Eng. Aspects* 207: 139-145
[28] Protein Data Bank, http://www.rcsb.org/pdb.
[29] Hecht, E. (2002) *Optics*. 4th ed., Pearson Addison-Wesley, Boston, USA.
[30] Spaeth, K., A. Brecht, and G. Gauglitz (1997) Studies on the biotin–avidin multilayer adsorption by spectroscopic ellipsometry *J. Colloid Interface Sci.* 196: 128-135.

[Received February 20, 2004; accepted April 9, 2004]