Extended Abstracts of the 2010 International Conference on Solid State Devices and Materials, Tokyo, 2010, pp537-538

Detection of Antigen-Antibody Reaction Using Si Ring Optical Resonators Functionalized with an Immobilized Antibody-Binding Protein

Mizue Nishida[1], Masataka Fukuyama[1], Yosuke Abe[1], Yoshiteru Amemiya[1], Takeshi Ikeda[1], Akio Kuroda[2], and Shin Yokoyama[1]

[1]Research Institute for Nanodevice and Bio Systems, Hiroshima University
[2]Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University
1-4-2 Kagamiyama, Higashihiroshima, Hiroshima 739-8527, Japan
Phone: +81-82-424-6265 Fax: +81-82-424-3499 E-mail: fukuyama-m@hiroshima-u.ac.jp

1. Introduction

Recently high-sensitive compact biosensors using Si microring optical resonators have been attracting great attention [1]. We propose the integrated biosensor chip using Si ring resonators, where different receptor is immobilized on each sensor (Fig. 1). Signal detection is carried out by the matrix of light-input and detection waveguides, which are respectively connected to laser diodes and photodetectors. The Si rings are arranged at the cross points. The unique point of our work different from Vos *et al*. [1] is to use the silicon-binding protein (designated Si-tag), which binds to SiO_2 surface, as an anchoring molecule to immobilize bioreceptor on the Si rings in an oriented manner (Fig. 2) [2-4]. In the integrated biosensor chip, many kinds of Si-tag-receptor fusions are required for high-throughput detection of analyte. So far, the Si-tag-receptor fusions were prepared by the recombinant DNA and protein expression technique [3, 4], which is time consuming and may be not suitable for preparing many kinds of receptors. In contrast, it is known that the protein A binds to many kinds of mammalian antibodies only by mixing the antibody solution [5]. In the previous paper [2], we constructed the fusion protein of Si-tag and protein A (Si-tagged protein A) for rapid immobilization of various kinds of antibodies (Fig. 3). In this paper, the Si ring biosensors were functionalized with various antibodies using the Si-tagged protein A as an intermediate binder, and the label-free detection of antigen have been achieved.

2. Experimental

The principle of the Si-ring biosensor is based on the change in the resonance wavelength induced by adsorption of some substance on the ring surface. Si ring resonators were fabricated by conventional electron-beam lithography and dry etching on silicon-on-insulator substrate (Fig. 4). The Si-tagged protein A was constructed by standard recombinant DNA and protein expression techniques as described previously [2]. The measurement setup is shown in Figs. 5 and 6. The details were reported in Refs. 3 and 4. The measurement procedure is shown in Fig. 7. Briefly, the ring is immersed in the solution containing the Si-tagged protein A (b), and then immersed in the antibody (mouse antibody subtype IgG_{2a}) solution (c). After that, the rings are immersed in antigen solution (d). The resonance spectra have been measured at each step. In this study, two proteins, green fluorescent protein (GFP) and prostate specific antigen (PSA), a specific diagnostic marker for the prostate cancer, were used as model antigens.

3. Results and Discussion

3.1. Immobilization of antibody using Si-tagged protein A

The resonance wavelength shifted as the reaction step proceeded from (a) to (d) in Fig. 7. With the adsorption of Si-tagged protein A on the Si ring (b), the resonance-wavelength shift saturates at ~0.3 nm (not shown). Next the antibody binds with the protein A (c). The reaction behavior as a function of the antibody concentration is shown in Fig. 8. It is found that the resonance-wavelength shift (Fig. 8(b)) fits well to Langmuir's equation shown in the inset of the figure, suggesting that the reaction of the antigen with protein A saturates at one monolayer.

3.2 Antigen detection

In the next step, we have examined the binding of antigen to the antibody immobilized on the ring via Si-tagged protein A. First, the reaction between GFP and anti-GFP antibody was examined. The result is shown in Fig. 9, where the concentrations of GFP and anti-GFP antibody were both 10^{-4} g/ml. The resonance wavelength shifts as the reaction steps proceed, indicating successful detection of antigen-antibody binding.

Finally, we have investigated the behavior when the antigen concentration is changed. Figure 10 shows the results of PSA detection using anti-PSA antibody. The sample was first saturated with the excess amount of anti-PSA antibody and Si-tagged protein A. Then it was exposed to the solution containing PSA followed by the washing in 20 mM Tris-HCl buffer and the measurement of resonance spectrum. This process was repeated from low PSA concentration to high concentration for the same sample. At the PSA concentration less than 10^{-7} g/ml, the resonance wavelength shifts toward shorter wavelength. On the other hand, at the high concentrations ($> 10^{-6}$ g/ml) it shifts to longer wavelength. This interesting behavior is explained by the model shown in Fig. 11. There exist two kinds of reactions: (1) dissociation of anti-PSA antibody from protein A and (2) binding of PSA to anti-PSA antibody. At the low PSA concentration the dissociation may be dominant and at the high concentration the binding may be dominant. The sensitivity of antigen detection is found to be in the order of 10^{-6} g/ml. The practical biosensor requires the sensitivity of 10^{-9} g/ml, which will be possible by employing slot-type waveguide [6] and increase in the quality factor of the resonator.

4. Conclusion

We have developed the rapid functionalization method of Si-ring resonators with antibodies using Si-tagged protein A. Since various kinds of antibody can be used as receptors for biosensing, this method promises to realize the integrated biosensors for high-throughput analyte detection.

Acknowledgments

This work was partly supported by "Interdisciplinary Research on Integration of Semiconductor and Biotechnology at Hiroshima University" from the Ministry of Education, Culture, Sports, Science, and Technology of Japan and New Energy and Industrial Technology Development Organization (NEDO) of Japan.

References

[1] K.D. Vos *et al.*: Opt. Express **15**(2007)7610. [2] T. Ikeda *et al.*: Anal. Biochem. **385**(2009)132. [3] S. Yamatogi *et al.*: Jpn. J. Appl. Phys. **48**(2009) 04C188. [4] M.Fukuyama *et al.*: Jpn. J. Appl. Phys. **49**(2010)04DL09. [5] D.D. Richman *et al.*: J. Immunol. **128**(1982) 2300. [6] C.F. Carborg *et al.*: Lab Chip **10**(2010)281.

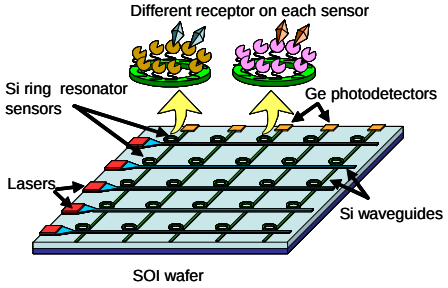


Fig. 1. Structure of integrated biosensor detectable plural kinds reactions rapidly.

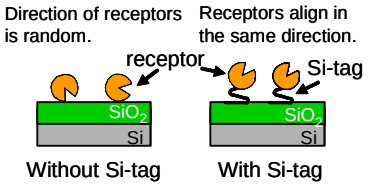


Fig. 2. Role of silicon binding protein (Si-tag).

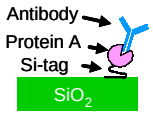
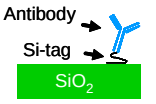
	Solution mixing easily generates antibody-protein A bond.
	Recombinant DNA and protein expression technique is required for each receptor (antibody).

Fig. 3. Role of protein A. Variety of receptors (antibodies) can be easily immobilized by using Si-tagged protein A.

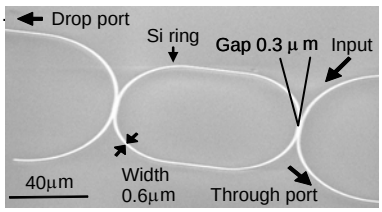


Fig. 4. Example of bird's-eye SEM photograph of fabricated Si ring resonator.

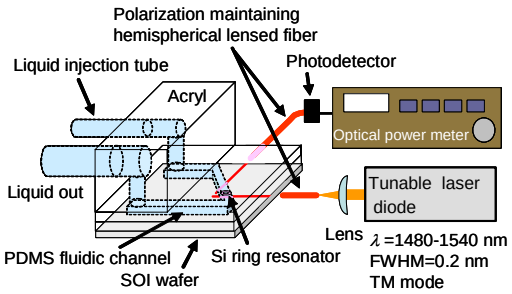


Fig. 5. Optical measurement system with fluidic channel, lensed optical fibers and optical microscope. PDMS is polydimethylsiloxane.

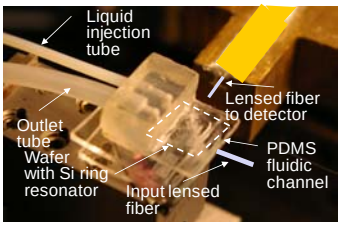


Fig. 6. Photograph of optical measurement system with fluidic channel, and lensed optical fibers.

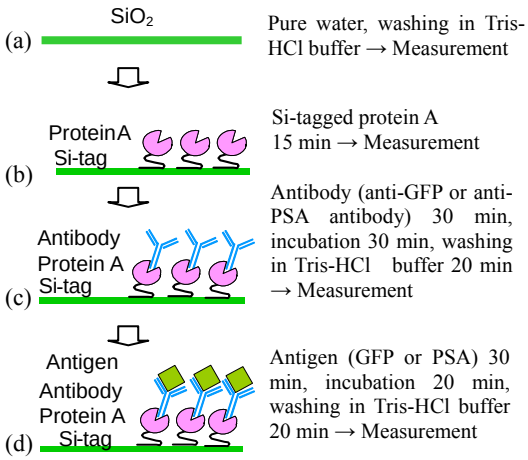
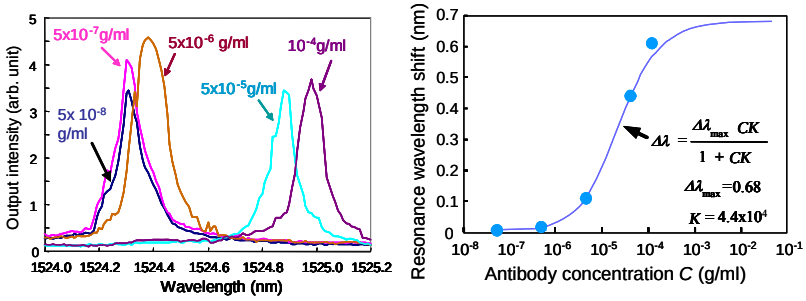


Fig. 7. Experimental procedure for detection of antigen and antibody reaction. "Measurement" means the measurement of optical resonance spectrum. Tris-HCl is $C_4H_{11}NO_3ClH$ for pH adjustment.



(a) (b)

Fig. 8. (a) Resonance spectra after each treatment step for antibody. (b) Concentration of antibody versus resonance wavelength shift and Langmuir's fitting curve.

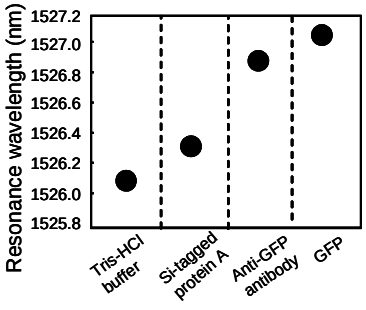


Fig. 9. Resonance wavelength shift after each treatment step for GFP and anti-GFP antibody reaction.

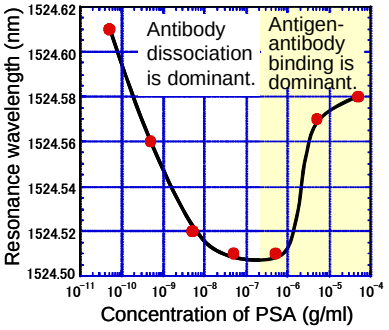


Fig. 10. Concentration of PSA versus resonance wavelength.

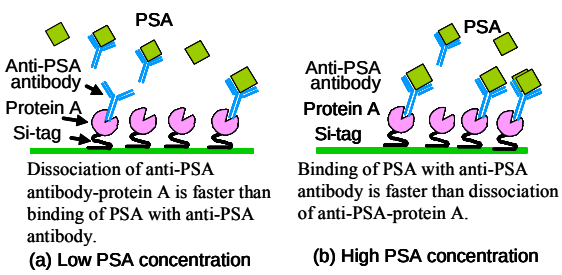


Fig. 11. Schematic model for the reaction at (a) low PSA concentration and (b) high PSA concentration.