

Sequential Detection of Immunoglobulin G via Nonspecific Adsorbed Staphylococcal Protein A Using PDMS Quartz Crystal Microbalance Sensor

PDMS 水晶振動子センサを用いた非特異吸着プロテイン A による免疫グロブリン G の連続捕捉

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1. Introduction

Recently, point-of-care testing (POCT) is attracting attention to reduce the burden on the patient and to improve the examination process. The main purpose of POCT is simple and quick examination at the bedside of the patient. Therefore, the studies about high performance analysis system for the reaction between the antigen and antibody, and so on, has been accelerated. In particular, the instruments (e.g., pump, filter, reactor) used in the protocol of biomolecule reactions, which is carried out at the hospital or laboratory, have been integrated. Consequently, they are miniaturized to a palm-size chip and also allow a series of analyzes on a chip. This integrated device is called lab-on-a-chip (LOC).^{1,2)} And it can be batch-produced and has high reproducibility because it is produced by the semiconductor microfabrication or nanoimprint technology. In recent years, the studies combined the different sensors such as quartz crystal microbalance (QCM) and surface plasmon resonance (SPR) to improve reliability have been investigated.³⁾ The QCM and SPR biosensor are the effective tools on the drug discovery for immunotherapy because they can evaluate the affinity of the antigen-antibody reaction quantitatively. Many studies on the miniaturization or sensitivity improvement of these sensors are reported, however, there is no report that the sensor and pump are integrated in a chip. In this study, the QCM biosensor chip, which is aimed at integration with the micro pump, was fabricated with poly(dimethylsiloxane) (PDMS). And then the performance as a biosensor chip was demonstrated.

2. PDMS QCM Biosensor

The PDMS is widely used as the base material or moving part of the micro pump because is silicone resin and flexible.⁴⁾ And it is also used as the substrate for LOC because it is possible to form the fine patterns of several tens nanometer order by

nanoimprint technology. The methyl group on PDMS surface is replaced with the hydroxyl group by the plasma treatment, therefore its surface changes to hydrophilic. When the PDMS substrates are adhered to each other after the plasma treatment, and then the covalent bond is achieved via oxygen atom. As a result, they are directly bonded. In this study, we focused on these useful characteristics of PDMS and developed the QCM biosensor chip fabricated with PDMS (TSE-3032, Momentive Performance Materials Inc.). **Figure 1** shows the schematic of the PDMS QCM biosensor chip with the experimental setup. The thin quartz oscillator (fundamental frequency 55.9 MHz in ultrapure water) is installed in the microchannel. All surfaces of the quartz oscillator are supported by the micropillars, semicircular walls, and stoppers without fixing mechanically. The sensor is operated by the copper foil antennas attached to the outside of chip. The quartz oscillator is excited by the electromagnetic wave applied from upper side antenna through inverse piezoelectric effect. And the electric potential induced on the quartz surface through piezoelectric effect is wirelessly detected by another antenna simultaneously. This wireless operation is performed using the superheterodyne spectrometer (RAM5000, RITEC, Inc.).

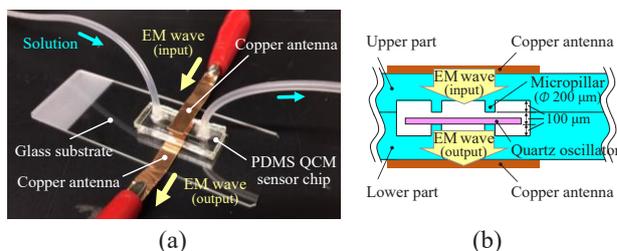


Fig. 1 Schematic of the PDMS QCM biosensor chip with the experimental setup: (a) experimental setup for the excitation and signal detection of the quartz oscillator with the EM wave, and (b) sensor chip cross-section.

3. Experimental procedure

Figure 2 shows the protocol performed in this study. (a) The staphylococcal protein A (SPA) solution (0.1 mg/ml) was injected in the microchannel with a pipette, and SPA molecule is adsorbed on the quartz surface nonspecifically. (b) Subsequently, after the rinse by ultrapure water injection, the bovine serum albumin (BSA) solution (10 mg/ml) was injected similarly. Thereafter, the tube was connected with the piezo pump (custom-made, Takasago Electric, Inc.), and then the quartz surface was rinsed by feeding ultrapure water as the buffer solution. (c) After the baseline of the resonance frequency change was stable, the rabbit-immunoglobulin G (R-IgG) (10 μ g/ml) solution as the target substance was injected and the binding curve was obtained. And the dissociation curve was then obtained by the rinse. (d) Thereafter, the glycine-HCl buffer (GHB) solution was injected to dissociate the specific binding between the SPA and R-IgG, and then the rinse was performed. After the baseline was stable again, the R-IgG was injected similarly. The protocols from (c) to (d) were continuously performed eight times.

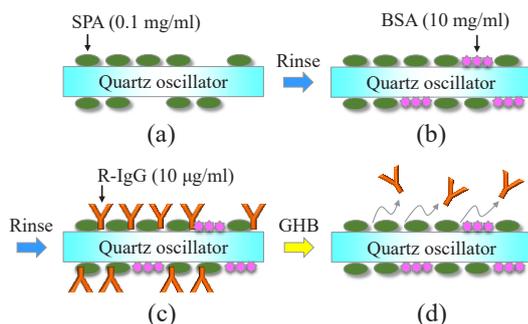


Fig. 2 Bioassay carried out in this study: (a) nonspecific adsorption of SPA, (b) blocking with BSA, (c) binding reaction of R-IgG via SPA, and (d) dissociation of R-IgG by GHB injection.

4. Results and Discussion

Figure 3 (a) shows sequential detections of the R-IgG via the SPA. The binding and dissociation curves were obtained in eight experiments. Moreover, the dissociation curves became steady state without returning to baseline. This result indicates that the specific binding is sufficiently performed between the SPA and R-IgG. As shown in **Fig. 3 (b)**, for instance, about 13.7 ng of the R-IgG could be captured. After the GHB injection and the rinse, it was found that the R-IgG captured via the SPA had been sufficiently dissociated because the baseline returned to initial level. However, the eighth baseline level was higher than other baselines. It is assumed that the baseline shift was caused by peeling of the nonspecific adsorbed SPA. This issue that the receptor peels is

possible to improve by immobilizing it on the quartz surface deposited gold film via the self-assembled monolayer (SAM). However, the developed sensor chip has sufficient performance to analyze beside the patient simply and quickly similar to LOC. Furthermore, this sensor chip can be combined with the micro pump made of PDMS by the direct bonding, therefore the adding of value and miniaturization of analysis system are expected.

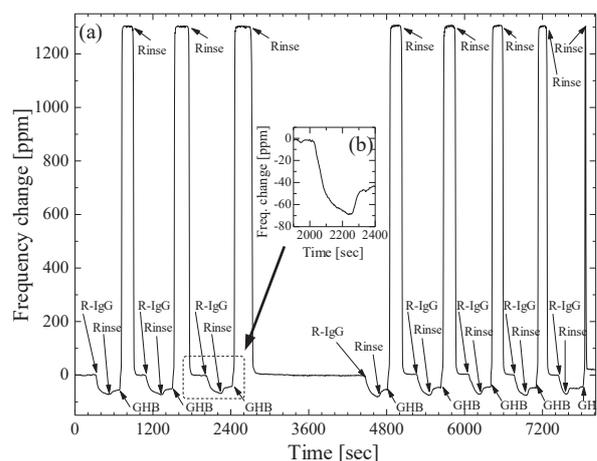


Fig. 3 Continuous detection of the R-IgG via the SPA adsorbed on the quartz surface nonspecifically: (a) binding and dissociation of the R-IgG performed eight times, and (b) reaction curve performed the third time.

5. Conclusion

The PDMS QCM biosensor chip was fabricated, and the sequential detection of the R-IgG via the SPA succeeded. This result indicates that the developed sensor chip will make the significant contribution to the adding of value and miniaturization of analysis system for POCT in future.

Acknowledgment

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