3Pa3-5

Proceedings of Symposium on Ultrasonic Electronics, Vol. 32 (2011) pp. 359-360
8-10 November, 2011

Development of MEMS Quartz Crystal Microbalance Biosensor with an Electrodeless Embedded Quartz Resonator

無電極水晶内蔵型 MEMS-QCM バイオセンサの開発

Shintaro Nishikawa[1‡], Fumihito Kato[1], Taiji Yanagida[1], Hirotugu Ogi[1], and Masahiko Hirao ([1]Graduate School of Engineering Science, Osaka University.)

西川 慎太郎[1‡], 加藤 史仁[1], 柳田 泰次[1], 荻 博次[1], 平尾 雅彦[1] ([1]大阪大学 大学院基礎工学研究科)

1. Introduction

The earlier works proved that if one gets a disease, the corresponding specific protein is secreted [1]. Diagnostic with a biosensor therefore attracts significant attention for early detection of diseases and their effective treatments.

Additionally, it has been recognized that some of specific proteins cause diseases. Finding such an antibody to show a high affinity for these toxic proteins contributes to develop the effective drugs. In this case, measurement of the affinity between the antibody-antigen reaction is required.

We consider that the quartz crystal microbalance (QCM) can be a candidate biosensor for satisfying these issues. QCM has the ability to measure the mass of adsorbed substances attached on the surface of the quartz crystal through the change in its resonance frequency. However, QCMs have too low sensitivity to be used for diagnosis. Thus, significant improvement in the sensitive has been needed in the QCM study.

It has been obvious that the sensitivity of QCM is dependent on thickness of the quartz. Using thinner quartz corresponds to higher sensitivity assay [2]. For the typical QCM, a few hundred nanometer-thick electrodes are attached to the quartz surfaces to oscillate quartz. In this situation, thinning the quartz enhances the dynamic mass of the electrodes. Therefore, it has been never straightforward to improve the QCM sensitivity. Moreover, we use a strong acid to wash the flow channel and the quartz, which peels the electrodes.

As a breakthrough to these problems, we have developed the resonance acoustic microbalance with naked embedded quartz (RAMNE-Q) with a MEMS process.

2. Fabrication of RAMNE-Q chip

The RAMNE-Q chip has the trilaminar structure (glass-silicon-glass) as shown in Fig. 1. The Si microchannel is manufactured by an inductive coupled plasma reactive ion etching (ICP-RIE) into the silicon substrate. The width of Si microchannel is 300 μm. The bare quartz resonator (29.5 μm thick) is embedded in the microchannel with being supported by silicon micropillars (100 μm in diameter). In this case, the quartz crystal softly contacts with the micropillars. The glass substrates are bonded to upside and downside of silicon substrate by the anodic bonding method.

We apply oscillating electric field to the quartz resonator by line antennas. The third-order resonance mode (~ 170 MHz) was used throughout this study. The Q value is about 30000 in air and 1000 in the solution flow. As the result, we achieve monitoring of the resonance frequency of the embedded quartz without contact from outside.

The RAMNE-Q accrues many advantages compared with the typical one. First, we can use both surfaces of the quartz resonator as detection area. Second, improvement of the sensitivity is easily achieved by thinning the quartz resonator. Finally, the RAMNE-Q chip can be repeatedly used by washing with strong acid. Thus, the QCM we have developed in this work is an innovative biosensor.

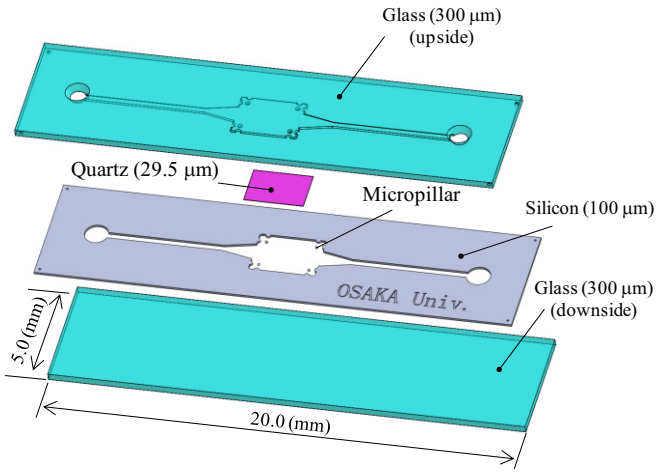

Fig. 1 Schematic of the RAMNE-Q chip.

3. Experimental procedure

We detected human immunoglobulin G (hIgG) via staphylococcal protein A (SPA) immobilized on both surfaces of the quartz nonspecifically at 25 °C as the performance test of the QCM we developed in this work.

At first, phosphate buffered saline (PBS) solution was flowed at a flow rate of 200 μl/min. After the resonance frequency becomes stable, the SPA solution (400 μg/ml in PBS) was injected to immobilize SPA molecules on the both surfaces of

the quartz. Then, hIgG solutions (a variety of concentrations in PBS) were injected continuously for 25 min. After each binding reaction between SPA and hIgG, Glycine-HCl buffer (GHB) (pH 2.2) was injected to dissociate hIgG from SPA.

4. Result and Discussion

We show the resonance frequency change during the injection sequence in Fig. 2(a). Fig. 2(b) shows the closeup of the binding reaction curves for injections of 10 and 1 ng/ml hIgG solutions. The resonance frequency change is 5.51 kHz by the nonspecific adsorption of SPA, corresponding to adsorption of 10.1 ng of SPA. When each hIgG solution was injected, the resonance frequency decreased even for a low-concentration 1 ng hIgG solution. When we injected GHB, the resonance frequency rose, indicating dissociation of SPA and hIgG. Moreover, Fig. 2 demonstrates clearly the repeatable use of the QCM.

The resonance frequency change for first injection of 10 μg/ml hIgG solution is larger than the latter. We attribute this to the nonspecific adsorption of hIgG molecules on uncovered surface area of the quartz.

We carried out fitting of exponential curve to each reaction curve and found good correlation between the exponential coefficient α and hIgG concentration (Fig. 3), indicating that quantitative determination of the analyte concentration is made possible by measuring the exponential coefficient/

5. Conclusion

In this work, we developed a quartz crystal microbalance with electrodeless quartz resonator embedded in Si microchannel. We succeed in detection hIgG via SPA immobilized on both surfaces of the quartz many times, and monitoring the resonance frequency change in real time, even at the low concentration hIgG (1 ng/ml). Therefore, this RAMNE-Q can be applied diagnostic and affinity assay to study interaction of biomolecules. The mass sensitivity will be improved more by embedding thinner quartz plate.

Reference

1. M. Forman, J. Trojanowski, and V. Lee, Nature Medecine, 10, 1055(2004).
2. G. Sauerbrey, Zeitschrift fur Physik, 155, 206(1959).

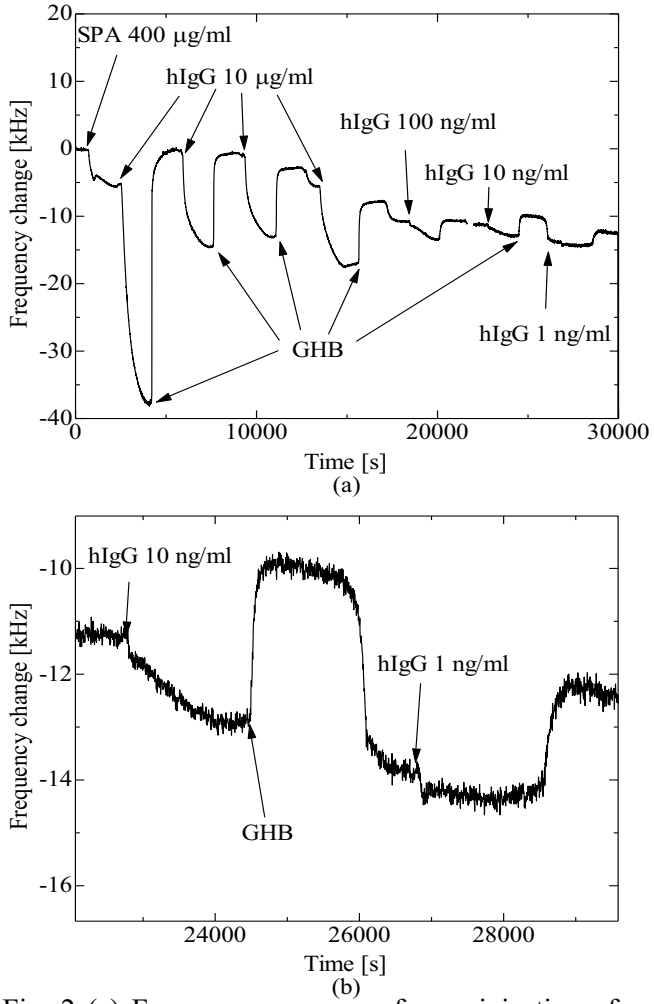

Fig. 2 (a) Frequency responses for an injection of 400 μg SPA solution and a variety of concentration hIgG solution. (b) Closeup of frequency responses caused by injections 10 ng/ml and 1 ng/ml hIgG solution in (a).

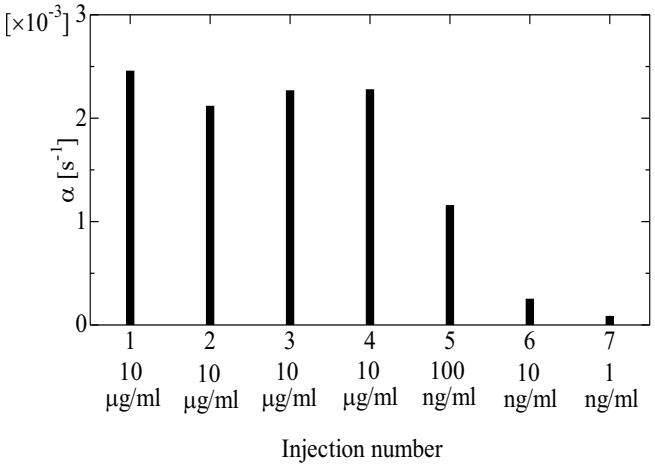

Fig. 3 Comparison of exponential coefficient for each reaction curve.