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Development of a High-Frequency Electrodeless Quartz Crystal Microbalance Chip with a Bare Quartz Resonator Encapsulated in a Silicon Microchannel and Its Application to a Biosensor

シリコン微細流路カプセル型ベア水晶内蔵無電極高周波 QCM チップの開発とその応用

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1. Introduction

The early stage detection of the disorder is enabled by detecting specific protein secreted after the attack of disorders as marker proteins[1)]. The fluorescence labeling technique is well known in the detection of such marker proteins[2)]. However, the labeling technique produces some problems, such as influences on structures and functions of the protein and obstruction to the binding reaction by the labeling material, in biochemical reactions. Therefore, the demand of a labeling-free biosensing method has risen.

On the other hand, in a peculiar protein to the disorder, the protein itself also becomes a disorder factor. Therefore, developing the antibody medicine with high affinity for these antigenic proteins enables more effective immunotherapy. In this case, it is necessary to measure the biochemical reactions to evaluate the affinity in real time precisely.

As a method to improve these problems, the quartz crystal microbalance (QCM) biosensor is known, which has the ability to measure quantitatively the amount of mass adsorptions and monitor the binding reaction in real time without any labeling. QCM has shown pronounced ability for studying recognition behavior among biochemical molecules through changes in resonance frequencies of the quartz plate. The sensitivity significantly depends on the mass of the quartz resonator, and the more the mass of resonator decreases, the higher sensitivity QCM achieves[3)]. Therefore, it is necessary to use a thinner quartz resonator, which possesses a large adsorption area and is the lightweight, corresponding to higher frequency resonator, to develop a high-sensitive QCM. However, the thinner quartz resonator disturbs use in the liquid environments and may induce a breakage at handling, because the thin quartz resonator is fragile. As a breakthrough of such problems, we have developed a high-frequency electrodeless quartz crystal microbalance chip with a bare quartz resonator encapsulated in a silicon microchannel.

2. Sensor Description

The developed sensor is composed of three substrates (glass-silicon-glass) as shown in Fig.1. A silicon microchannel is fabricated by an inductively coupled plasma reactive ion etching (ICP-RIE) into the middle silicon substrate, and electrodeless bare quartz (t=9.6 μm) is embedded in the microchannel so as to be supported by micropillars (Φ100 μm) without direct contacts. The glass substrates are bonded to both sides of the silicon substrate by the anodic-bonding method.

The method of inducing a quasistatic electric field is used for the excitation and signal detection of the quartz resonator[4-6)]. As the result, the device achieves the noncontacting monitoring of the resonance frequency of the encapsulated quartz resonator from the outside.

The developed device is electrodeless, so that it can eliminate the unfavorable effects of the metal electrodes (e.g. inertial resistance), and it allows the use of the entire surface of both sides of the quartz resonator as the detection area. Supporting a quartz resonator with the micropillars can decrease the structural damping for the vibration energy. Consequently, the Q-factor can be high to achieve a high S/N ratio. The developed device improves fragility at handling and obstructions to the extensive use, particularly for the liquid environment, because a bare quartz resonator is encapsulated in a rigid silicon microchannel. Furthermore, the device can be reused semi permanently, because the whole surface of the quartz can be completely cleaned by flowing a strong acid solution such as a piranha solution in microchannel. Therefore, this devise doesn't need reassembly, and then the repeatability becomes

highly precise. This device is high sensitive to the mass loading effect because of the high fundamental resonance frequency (172 MHz) and allows to ignore the viscosity effect on the frequency change. Then, this device can be integrated with other analytical systems, because it is fabricated by micromachining technology.

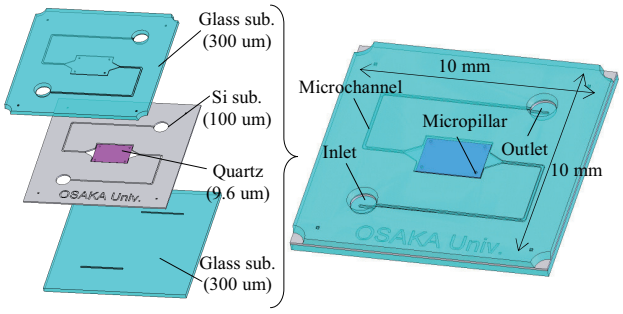

Fig.1 Schematic high-frequency electrodeless quartz crystal microbalance chip with a bare quartz resonator encapsulated in a silicon microchannel

3. Experimental procedure

We demonstrated the usefulness and high sensitivity of the developed device by detecting a human immunoglobulin G (hIgG) via staphylococcal protein A (SPA) immobilized on both sides of quartz surface nonspecifically at 37ºC. At first, ultrapure water as buffer solution was flowed at a flow rate of 100 µl/min, and the output signal was ensured the baseline. Then, the SPA solution (400 µg/ml in ultrapure water) was injected to immobilize SPA molecules on both sides of quartz surface nonspecifically, and afterwards the surfaces were rinsed by flowing ultrapure water. hIgG solution (10 µg/ml in ultrapure water) was injected successively for the detection of the protein, and afterwards the quartz surfaces were rinsed by flowing ultrapure water. The resonance frequency was monitored during the injection sequence.

4. Results and Discussion

Fig. 2 shows the frequency response during the immobilization, rinsing, binding reaction, and rinsing procedures. Ensuring the baseline, the device showed the stability of several tens ppm. The frequency change due to the nonspecific adsorption of SPA was 34.6 kHz corresponding to adsorption of 21.8 pg of the SPA molecule. When the quartz resonator was rinsed with ultrapure water, the frequency change was not observed. Therefore, the rinsing hardly caused the exfoliation of SPA. In the flowing of the hIgG solution successively executed, the frequency change due to the adsorption of hIgG was 99.1 kHz corresponding to adsorption of 62.3 pg of the hIgG molecule. When the quartz resonator was rinsed with ultrapure water after the binding reaction, the frequency change was not observed.

These results show that the developed device operates as a biosensor that can monitor during the binding reactions in real time without any labeling.

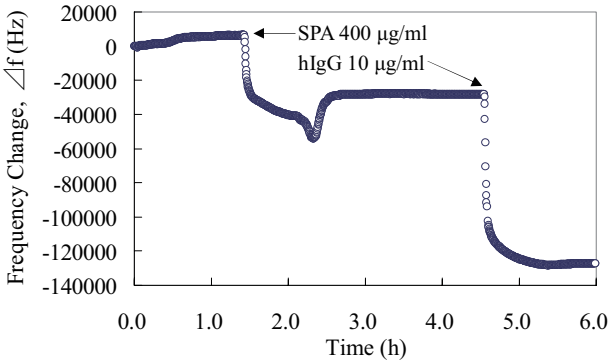

Fig. 2 Behavior of the frequency change caused by the injections of 400 µg/ml PSA solution and 10 µg/ml hIgG

5. Conclusion

We developed the high sensitive biosensor, which can monitor during the binding reactions in real time without any labeling, which can reuse semi permanently just to flow a strong acid solution and doesn't need reassembly. Using this biosensor, the hIgG was detected via the SPA immobilized on the quartz resonator nonspecifically.

References

1. M. H. F. Meyer et al.: Biosens. Bioelectron. **21** (2006) 1987.
2. Y. Lee et al.: Biosens. Bioelectron. **25** (2009) 332.
3. G. Z. Sauerbrey: Z. Phys. **155** (1959) 206.
4. H. Ogi et al.: Jpn. J. Appl. Phys. **44** (2005) 4381.
5. H. Ogi et al.: Jpn. J. Appl. Phys. **46** (2007) 4693.
6. H. Ogi et al.: Biosen. Bioelectron. **22** (2007) 3238.