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Development of RAMNE-Q Biosensor with MEMS Process for High-Frequency Measurements

MEMS プロセスによる高周波計測のためのラムネ Q バイオセンサの開発

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1. Introduction

The biosensor which makes early detection of the serious diseases possible is attracting attention as a simple diagnostic tool in point-of-care testing (POCT)[1, 2]. Among various biosensors, the quartz crystal microbalance (QCM) allows real-time monitoring of the biomolecular reaction between receptor and analyte proteins and evaluation of the thermodynamic parameters of the reaction. It is then a candidate of effective biosensor for POCT. The QCM biosensor is a mass-detection sensor, which detects the adsorbed mass as the resonance-frequency shift of the quartz resonator when the objective substance is captured by the receptor immobilized on the quartz surfaces operating at thickness shear vibration[3, 4]. The mass sensitivity of QCM is inversely proportional to the square of the quartz plate thickness; it is necessary to use thinner quartz plate to make the sensitivity higher sensitive effectively[5]. However, the conventional QCM needs heavy Au electrodes on the quartz surfaces for the vibration excitation and signal detection and for the immobilization of the self-assembled monolayer (SAM). Therefore the thinner quartz plate is, the more the inertial resistance by Au electrodes rises, and so vibration characteristics are deteriorated. Moreover, the structural damping is induced because the quartz resonator is fixed mechanically, and the quartz resonator is easy to break at the handling because the thin quartz plate is fragile.

As a breakthrough to these issues, we developed the resonance acoustic microbalance with naked-embedded quartz (RAMNE-Q) biosensor using the micro-electro-mechanical systems (MEMS) technology[6-8]. The RAMNE-Q is semi-permanently reusable, free from breakage of a quartz plate, and structural damping is remarkably low, because it is constructed of the three rigid substrates (SiO_2/Si/SiO_2) and a thin quartz resonator (9.3 μm~) supported by the micro pillars and circular walls without fixing is packaged in the microchannel. The RAMNE-Q, which is focused on high-affinity of nonspecific adsorption between the quartz surface and the protein molecules, is operated wirelessly through electromagnetic waves. Therefore, the quartz resonator installed in the RAMNE-Q chip is the electrodeless blank quartz plate, and then it is not influenced by the inertial resistance.

There is, however, a disadvantage in the RAMNE-Q biosensor we previously developed[6-8]; the mass detection area of the quartz plate was not able to be used effectively, because the flow velocity distribution was occurred in the narrow gap between the quartz plate side and silicon microchannel wall. In this study, we developed a new RAMNE-Q biosensor chip, which equips the wide silicon channel as a method to improve this issue.

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2. Experimental Setup

Fig. 1 shows the new version of the RAMNE-Q chip. The design concept is realization of the flow-velocity-distribution reduction with the wider silicon channel compared with the conventional RAMNE-Q.

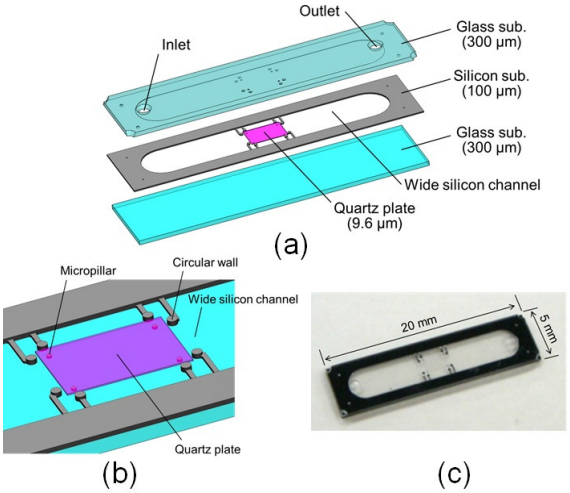

Fig. 1 (a) Stacks of the new RAMNE-Q with a wide silicon channel. (b) An enlarged view near the quartz resonator. (c) A photograph of the RAMNE-Q chip.

The basic characteristics of the new RAMNE-Q chip as a biosensor are evaluated, using the specific binding between streptococcal protein G (SPG) and

human immunoglobulin G (hIgG). The quartz resonator was excited wirelessly by the electromagnetic wave inputted from a flat antenna located, and the electromagnetic wave induced by the vibration of the quartz resonator was detected by the other antenna[7]. In this study, a standard network analyzer was applied to the excitation and detection of the quartz resonator. The phosphate buffered saline (PBS) solution was used as the buffer solution. The procedure of assay was following: The PBS solution was flowed in the wide silicon channel using a homemade micropump. The SPG solution (200 μg/mL in PBS) was injected to immobilize SPG molecules on the naked quartz surfaces nonspecifically after the baseline became stable. After this, the chip inside was rinsed by PBS solution. The hIgG solution (100 pg/mL in PBS) was then injected.

3. Results and Discussion

Fig. 2 shows a resonant spectrum of the new RAMNE-Q biosensor of the fundamental resonance frequency of 172 MHz. The quality factor was relatively high (>~400) despite the fact that the resonator was entirely immersed in the flowing solution. This result indicates that the vibration energy dispersion of a quartz resonator is extremely low because of the slight supports by the micorpillars and walls without fixing.

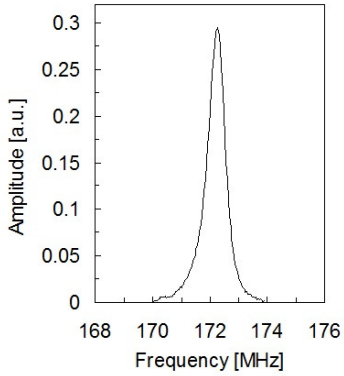

Fig. 2 An example of the resonant spectrum of the RAMNE-Q with a wide silicon channel.

Fig. 3 shows examples of the real-time monitoring through the assay sequence: the nonspecific adsorption of the SPG molecules and the binding reaction between the hIgG and SPG. The frequency change due to the nonspecific adsorption of the SPG was 240 ppm (corresponding to 25.9 ng of SPG molecule), and then the frequency change due to the buffer solution caused small recovery, indicating the high affinity between the protein and the quartz surfaces even with the nonspecific adsorption. (This nature of a RAMNE-Q biosensor realizes a replacement-free biosensor.) Subsequently, a significant frequency change due to the specific adsorption between the hIgG and SPG was found to be 4.7 ppm at 5000 s (corresponding to 0.51 ng of hIgG molecule). This result indicates that the new RAMNE-Q biosensor has the ability to detect 100 pg/mL hIgG solution via the SPG molecules immobilized on the naked quartz surfaces nonspecifically.

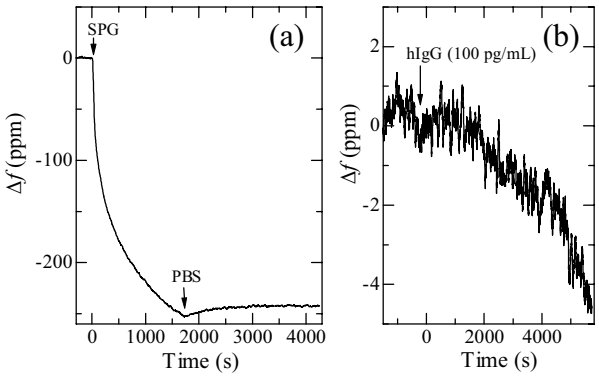

Fig. 2 (a) Frequency response by injection of the SPG solution (200μg/mL) and PBS rinsing. (b) Binding curve for injection of the hIgG solution (100 pg/mL) after sequence (a).

4. Conclusion

We developed a new RAMNE-Q chip, which has the wider silicon channel, and succeeded in the demonstration of the chip as a biosensor through the evaluation of a resonant spectrum and the detection of the hIgG molecules via the SPG molecules immobilized on the quartz surfaces nonspecifically. The RAMNE-Q biosensor being a high value-added biosensor opens up new possibilities for the QCM biosensor.

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