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Development of 170-MHz Wireless-Electrodeless Quartz Crystal Microbalance Biosensor

170 MHz 無電極 QCM バイオセンサの開発

Hironao Nagai[1‡], Yuji Fukunishi[1], Hirotsugu Ogi[1,2], Masahiko Hirao[1] and Masayoshi Nishiyama[3] ([1]Graduate School of Engineering Science, Osaka University, [2]Life Phenomena and Measurement Analysis, JST PRESTO, [3]Renovation Center of Instruments for Science Education and Technology, Osaka University)

長井大尚[1‡], 福西勇志[1], 荻博次[1,2], 平尾雅彦[1], 西山雅祥[3]（[1]大阪大 基礎工, [2]JST さきがけ, [3]大阪大科学技術教育機器リノベーションセンター）

1. Introduction

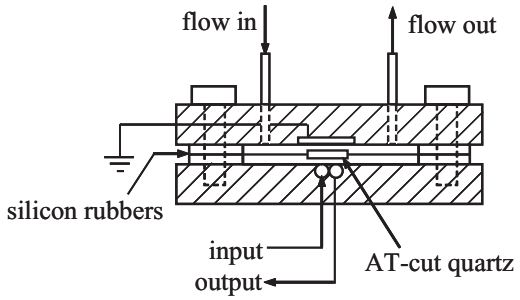
The quartz crystal microbalance (QCM) biosensor can quantitatively detect specific protein markers, which are excreted with corresponding disorders, such as amyloid-β for Alzheimer's disease[1)] and glypican-3 for hepatocellular carcinomas[2)]. Their detection in the early stage highly contributes to the effective treatment of the disorder. The QCM biosensor also possesses an important ability of monitoring biochemical reactions, such as the antigen-antibody reaction, in real time, leading to quantitative evaluation of the affinity between proteins. This contributes to the screening in searching an antibody for a target antigen in the drug development process. Thus, by increasing the sensitivity of the QCM biosensor, it significantly contributes to the diagnosing and drug development.

The sensitivity of QCM is improved by thinning the thickness of the quartz crystal because it is a mass-sensitive biosensor. However, in a conventional QCM biosensor, gold electrodes deposited on both sides deteriorates the sensitivity because of the large inertial resistance due to much higher mass density than that of quartz. Thus, the sensitivity of the QCM is considerably improved by eliminating the gold electrodes on the quartz crystal.

We have developed a wireless-electrodeless (WE) measurement technique and achieved a high frequency QCM with the fundamental resonance frequency of 55 MHz[3)]. Here, we intend to develop a much higher frequency WE-QCM system using a 10-μm-thick AT-cut quartz crystal, whose fundamental frequency is near 170 MHz. We excite and detect pure shear vibration of the quartz crystal using a line antenna. In this method, the quartz crystal can be used semipermanently because of the elimination of the electrodes on both sides of the quartz crystal. Moreover, the viscosity effect on the frequency change becomes negligible at higher frequencies. Here, we demonstrate the high sensitivity and usability of the 170 MHz WE-QCM by detecting a human immunoglobulin G (hIgG) via staphylococcal protein A (SPA) immobilized on both sides of quartz crystal surface nonspecifically.

2. Measurement Procedure

Fig. 1 shows the cross-sectional diagram of the homebuilt QCM cell. The 10-μm-thick quartz crystal is sandwiched by silicon rubber gaskets. The antenna[4)] consists of two straight-line copper wires, embedded in the bottom wall of the cell, and a thin copper film, embedded in the upper wall of the cell, for grounding. The tone-bursts voltage is applied to the excitation wire to induce the quasistatic electric field between the excitation wire and the thin film, and generate the pure shear vibration through the converse piezoelectric effect. After the excitation, the detection wire detects the reverberating vibration of the crystal through the piezoelectric effect. The resonance spectrum is obtained by sweeping the driving frequency and acquiring the amplitude as a function of the frequency. The resonance frequency is determined by a Gaussian function fitting. This procedure needs 10 s, and after the resonance frequency becomes stable, we monitored the phase of the detection signal at the fix frequency for quicker measurement (~0.01 s),



Email:ogi@me.es.osaka-u.ac.jp

and determined the resonance frequency using the linear relation between the frequency and the phase near the resonance frequency.

3. Experimental Section

It is known that SPA directly absorb on the naked quartz crystal surface[5], and by utilizing this nonspecific binding ability, SPA can be immobilized on the quartz crystal without any specialized treatments. The crystal was cleaned in piranha solution (98% H_2SO_4 : 33% H_2O_2 = 3.5 : 1.5). After rinsing with ultrapure water, it was immersed in phosphate buffer solution (PBS; pH 7.0) containing 0.4 mg/ml SPA for 24 h at 4ºC and rinsed by PBS. The crystal was then set into the homebuilt flow injection system for continuous and stable monitoring of the resonance frequency. The temperature in the cell was maintained at 25±0.1 ºC. The carrier solution was PBS, and the flow rate was 0.5 ml/min. The solution to be injected was selected by an automatic selector valve. After the resonance frequency was stable during the PBS flow, the hIgG solution was injected. When the resonance frequency reached an equilibrium state, a glycine-HCl buffer (0.1 M, pH 2.2) was injected to dissociate the hIgG molecules from SPA, which was followed by the injection of PBS. This injection sequence was repeated for solutions of various hIgG concentrations.

4. Results and Discussion

Fig. 2 shows the frequency responses to the injections of the hIgG solutions detected using three different QCMs with fundamental resonance frequencies of 5, 54 and 170 MHz. The 5 MHz QCM is a conventional QCM, but the other two are wireless-electrodeless QCM we developed. As shown **Fig. 2**, the change in the resonance frequency to the same hIgG solution drastically increases as the fundamental resonance frequency becomes higher. Especially, the 170 MHz QCM shows the amount of the frequency change larger than that of the conventional QCM by a factor of 1000. This is supported by the theory, where the resonance frequency change is proportional to the square of fundamental resonance frequency[6]. Also, at higher frequencies, the viscosity effect becomes insignificant compared with the mass loading effect[7], yielding more quantitative analysis. Thus, the 170 MHz QCM biosensor is superior to the conventional one not only in the sensitivity but also in the quantitative ability.

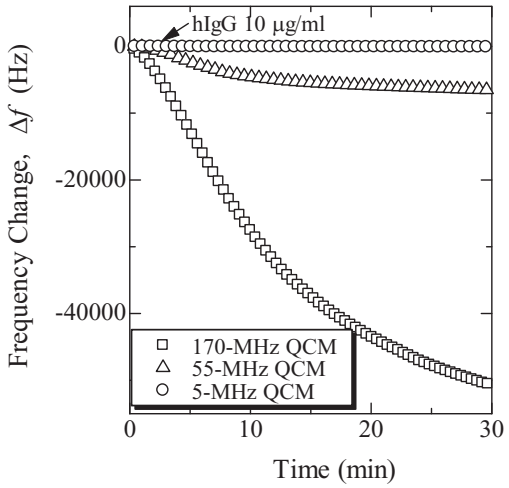


Fig. 2 Comparison of the frequency responses for injections of 10 μg/ml hIgG solution among three QCM biosensors.

5. Conclusion

We developed a high-frequency wireless-electrodeless QCM biosensor with the fundamental frequency of 170 MHz. A 10-mm-thick AT-cut quartz crystal was excited and detected by the noncontacting manner using the line antenna. Using this QCM, the hIgG was detected via the SPA immobilized on the quartz crystal nonspecifically. This QCM system showed the sensitivity higher than the conventional 5 MHz QCM by three orders of magnitude.

References

1. M. Forman, J. Trojanowski, and V. Lee: Nat. Med. **10** (2004) 1055.
2. M. Capurro, I. R. Wanless, M. Sherman, G. Deboer, W. Shi, E. Miyoshi, and J. Filmus: Gastroenterology **125** (2003) 89.
3. H.Ogi, K. Motohisa, K. Hatanaka, T. Ohmori, M. Hirao, and M. Nishiyama: Jpn. J. Appl. Phys. **46** (2007) 4693.
4. H. Ogi, Y. Fukunishi, T. Omori, K. Hatanaka, M. Hirao, and M. Nishiyama: Anal. Chem. **80** (2008) 5494.
5. H. Ogi, Y. Fukunishi, H.Nagai, K. Okamoto, M. Hirao, and M. Nishiyama: Biosens. Bioelectron. **24** (2009) 3148.
6. G. Z. Sauerbrey: Z. Phys. **155** (1959) 206.
7. K. K. Kanazawa: Faraday Discuss. **107** (1997) 77