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Study on optimal parameter for evaluating performance of targeted microbubbles using quartz crystal microbalance

水晶共振子を用いた分子標的マイクロバブルの性能評価システムにおける最適指標の検討

Ryosuke Shimoya[1†], Yasuhiro Yokoi[1], Kenji Yoshida[1, 2], Yoshiaki Watanabe[1] ([1]Doshisha Univ.; [2]Chiba Univ.)
下谷　遼資 [1†]　横井　康弘 [1]　吉田　憲司 [1, 2]　渡辺　好章 [1] ([1] 同志社大学　[2] 千葉大学)

1. Introduction

In recent years, technology for targeting microbubbles to specific molecules have been reported[1]. The microbubble is expected to be applied to drug carrier in drug delivery system since it can contain drug into the shell or inside. Targeted microbubble improves the efficiency of contrast and drug delivery. Actually, tumor in a rat is imaged selectively *in vivo*[2]. We aim to construct an *ex vivo* system to evaluate quantitatively the amount of specific attachment and reaction speed between bubbles and targeted cite. They are related to the amount of such drug. We propose the method using quartz crystal microbalance (QCM); thickness shear mode resonator. This report studies optimal parameter (e.g. resonant frequency of a QCM) for evaluating the perfomance of targeted microbubble .

2. Resonant characteristic of a QCM

A QCM is configured with electrodes on both side of AT-cut quartz. Due to the piezoelectric property, the quartz shows oscillation of thickness shear mode by applying voltage. The change in the resonant frequency f_s is caused by mass loading on the electrode as shown by Sauerbey[3]. Then, it is widely used as a biosensor. Q factor is a parameter related to a loss of oscillation energy of a QCM due to viscosity. Several researcher measure the resonant frequency and Q factor to observe the attachment of substance. It was demonstrated that frequency f_s and Q factor are affected by both mass loading and viscosity. On the other hand, frequencies of f_1 and f_2 were determined where the conductance of a QCM is half of maximum. These frequencies are also changed with the resonant frequency. Frequency f_2 especially is known as the one which is not affected by viscosity [4]. Frequency f_2 may enable the separated measurement of density and viscosity.

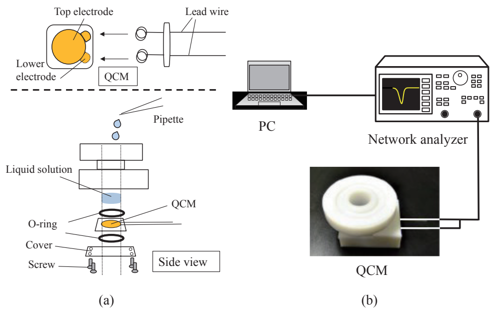


Fig. 1 (a) Structure of the quartz crystal microbalance (QCM) sensor. (b) Experimental system.

3. Trapping of microbubbles

Biotinylated microbubbles' suspension (BB) were prepared as targeted microbubbles. Biotin can bind to streptavidin specifically. Streptavidin molecules could be layered as targeted spot on the biotinylated self-assembled monolayer which spontaneously coats gold electrode of the QCM. Since streptavidin has four binding spots for biotin, biotinylated microbubbles should be trapped on the streptavidin layer.

For control experiment, the resonant characteristic of QCM was checked when control non-biotinylated microbubbles' suspension (CB) and biotinylated lipid solution (LI) as main component of BB's shell were also dropped on QCM. The comparison of results in case of BB and CB can determine the effect of the specific adsorption. To recognize the adsorption of bubble, BB was compared with LI including no bubbles.

4. Measurement system

Figure 1 shows experimental system and a QCM (QA-A5 M-AU(M)(SEP), Seiko EG&G) used in this experiment. The conductance of the QCM was measured with a network analyzer (E5071B, Agilent Technologies). The resonant frequency f_s where the conductance reached its maximum value and the two frequencies f_1, f_2 ($f_1<f_2$) where the conductance was half its

maximum were determined. Q factor was calculated from $f_s/(f_2-f_1)$. The QCM used in experiment has electrodes of 5 mm in diameter and fundamental frequency 5 MHz. In this report, the conductance in vicinity of the third harmonic frequency 15 MHz was measured. As an example, figure 2 shows the conductance of a QCM under the attachment of BB and CB.

5. Frequency change of a QCM due to bubbles

The effect on the resonant characteristic of a QCM due to specific adsorption of microbubbles is not reported. Microbubble may give different effect from usual substance because of the internal gas with diameter of several micron. We assume that the effect of bubble appear as the change of apparent viscosity. As above, f_2 is insensitive to viscosity. In contrast, f_1 is largely affected by viscosity. Measurement with these specific frequencies should provide beneficial information on the adsorption of bubbles. Compared with general measurement using f_s and Q factor, optimal measurement method for the evaluation was considered.

Figure 3(a) shows the shift of the resonant frequency f_s and the change of Q factor. Compared BB with CB, great shift of the resonant frequency by BB implies the specific adsorption. From the change of Q factor, the specific adsorption cannot be distinguished from the non-specific adsorption although there is a difference of the change. Compared BB with LI, the effect of bubbles appears in both f_s and Q factor.

Figure 3(b) shows the frequencies f_1, f_2 shift. The change of f_2 by LI and BB is larger than that of CB. These changes of f_2 represent the specific adsorption of a substance. f_2 was changed in almost the same amount between BB and LI. In contrast, Δf_1 due to microbubbles (BB) is larger than Δf_1 due to LI. This fact suggests that $\Delta f_1-\Delta f_2$ enables to judge whether bubbles exist or not. In other words, Δf_1 includes information on the bubbles. These results suggest that Δf_2 and $\Delta f_1-\Delta f_2$ are more optimal parameter in evaluation performance of bubbles' targeted ability.

6. Conclusion

We measured the several frequencies under dropping of microbubbles. The measurement of frequencies f_1, f_2 is optimal for the evaluation performance from the point of view that the adsorption of bubbles can be recognized. If the mechanism is understood, the detail information probably can be obtained by this measurement system.

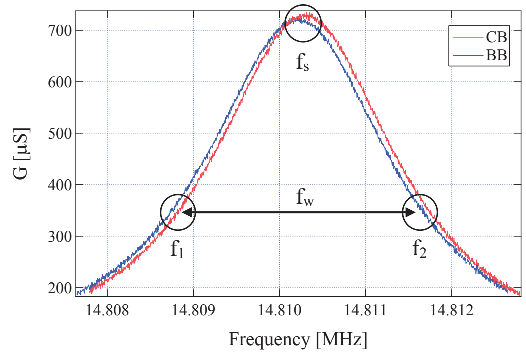
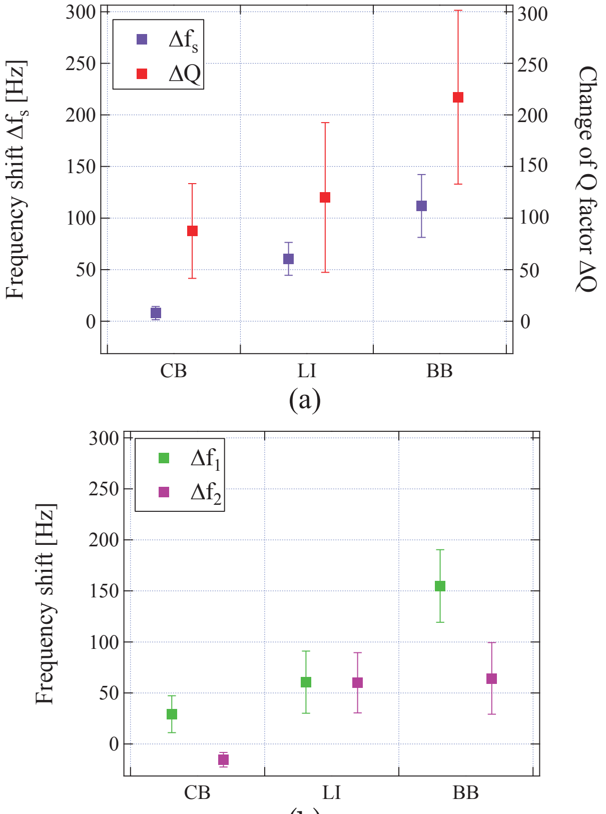


Fig. 2 Frequency characteristic of the conductance in vicinity of the third harmonic after the dropping control bubbles (CB) and biotinylated bubbles (BB).



(a)

(b)

Fig. 3 Shift amount of (a) resonant frequency f_s and Q factor, and (b) frequencies f_1, f_2 due to control bubbles (CB), biotinylated lipid (LI), and biotinylated bubbles (BB).

Acknowledgment

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