Proceedings of Symposium on Ultrasonic Electronics, Vol. 37 (2016)
16-18 November, 2016

# Development of sensor system using quartz crystal microbalance for detecting nano- and microbubbles
# 水晶共振子を用いたマイクロ・ナノバブル検出システムの構築

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

## 1. Introduction

Targeted micro- and nanobubbles can be applied as contrast agents for ultrasound moleculer imaging, where bubbles with ligand specifically bind to the target molecule. Several *in-vivo* studies demonstrated that bubbles targeting such as VEGFR2 and avb3 integrin were useful to monitor antiangiogenic or cytotoxic responses. To put the ultrasound moleculer imagng to practical use, several issue have remained. One of the most important issue is quantification of speciffically-bounded bubbles.

This study aims to understand the affinity between bubbles and target moleculer using a QCM system. We belive that the finding in this *in-vitro* experiment will be helpful to quantify the speciffically-bounded bubbles in *in-vivo* experiment. Previously we demonstrated that QCM enabled to monitor the adsorption of biothinylated bubbles to the streptavidin by monitoring the change of resonant frequency and Q-value of QCM[(1)]. In additon, it was revealed that the response of QCM was significantly affeceted by the amount and size disstribution of bounded bubbles[(1,2)]. This report investigates how the QCM response under the interation between bubbles and target molecule (streptavidin) depends on their concentrations.

## 2. Methods

A well-type cell is attached to an AT-cut QCM (QA-A5 M-AU(M)(SEP), Seiko EG&G Princeton Applied Research) connected to a network analyzer (E5071B, Agilent Technologies). The frequency characteristics of the conductance and the susceptance of the QCM are measured and the resonant frequency are calculated. Although the QCM has a fundamental frequency of 5MHz, measurements are conducted using the third harmonic of 15MHz due to the electrical impedance matching. The system are semi-automatically controlled using Labview software.

The measurement is conducted in accordance with the following procedure. First, 30 μL of ultrapure water is dropped into the cell. The resonant frequency in this condition is the criterion value. Then, the ultrapure water is replaced by the equivalent amount of bubble suspension (PBS solution). After the replacement of liquid, the bubble starts to absorb to the target molecule on QCM surface. As a result, the resonant frequency of QCM decreases due to the loading of bubbles. The changes in resonant frequency are recorded every 5 s by the system. After a certain period of time, the bubble suspension is washed by dropping the ultrapure water again. The difference in resonant frequency before and after the interaction relates to the amount of bounded bubble. Fig.1 shows the typical change in resonant frequency under the interaction between the bubble and target molecule.

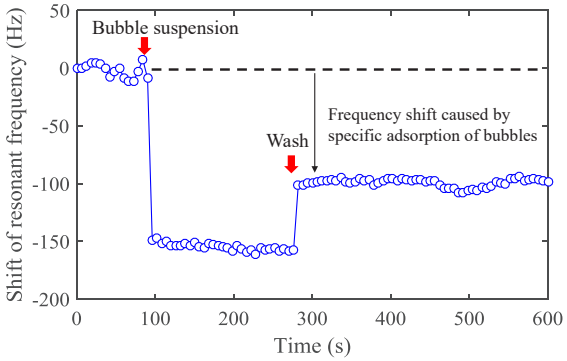



Fig. 1 Typical $\Delta f_b$-$t$ curve under interaction between bubble and target molecule. $\Delta f_b$ is the shift of resonant frequency of QCM.

## 3. Materials

Biotinylated microbubble suspension was prepared as targeted microbubbles. The bubbles consist of phospholipid (DSPC, DSPE-PEG2000, DSPE-PEG2000-biotin) and gas ($C_4F_8$: 7.94%, $N_2$: 92.06%). For the method of preparation of these microbubbles, refer to our previous paper[(1)]. The number density of bubbles in the suspension was evaluated by using a counting chamber. A streptavidin (Wako) layer was preliminary formed on the QCM electrode using a biotinylated self-assembled monolayer (Dojindo). Biotinylated microbubbles are attached through the streptavidin-biotin bond.

## 4. Results and Discussions

The amount of streptavidin on the QCM surface was controlled by diluting the solution of streptavidin in forming the layer. Before and after the formation of the layer, the resonant frequency of QCM was measured for quantifying the amount of streptavidin. Fig. 2 (a) shows the shift of the resonant frequency due to the formation of streptavidin layer as function of the concentration of streptavidin. It is found that the mass loading effect of streptavidin decreases the resonant frequency of the QCM. To check the state of streptavidin layer, it was observed by using scanning electron microscopy (SEM). Fig. 2 (b) shows the SEM image of QCM surface in different three conditions for the streptavidin concentration ($C_{\mathrm{avidin}}$). In case of $C_{\mathrm{avidin}}$=0.01 mg/mL, there are areas covered with streptavidin and naked area. In case of $C_{\mathrm{avidin}}$=0.1 mg/mL, all areas are covered with streptavidin although it is inhomogeneous.

It was investigated how the resonant frequency shift ($\Delta f_{\mathrm{b}}$) of QCM was affected by the number of bubbles in suspension. Fig. 3 (a) shows the $\Delta f_{\mathrm{b}}$–$N_{bubble}$ curve in different two conditions for concentrations of streptavidin solution ($C_{\mathrm{avidin}}$=0.0003 and 0.1 mg/mL), where $N_{bubble}$ is the number of bubbles in 30 μL of suspension. In both cases, it was found that the characteristic of $\Delta f_{\mathrm{b}}$–$N_{bubble}$ curve has two regions: unsaturated and saturated region. In the unsaturated regions ($N_{bubble} < 10^5$), the shift of resonant frequency logarithmically decreased. On the other hand, the shift was clearly saturated in case of $N_{bubble} > 10^5$. In all range for $N_{bubble}$, the frequency shift in case of $C_{\mathrm{avidin}}$=0.0003 mg/mL of streptavidin concentration was smaller than that in case of $C_{\mathrm{avidin}}$=0.1 mg/mL. Fig. 3 (b) shows $\Delta f_{\mathrm{b}}$–$C_{avidin}$ in the constant condition for $N_{bubble}$ ($N_{bubble} = 10^5$). Because $\Delta f_{\mathrm{b}}$ should be related to the amount of specifically-absorbed bubble, this suggest a possibility that the amount of target molecule could be estimated by counting the absorbed bubbles even in clinical situation.

## 5. Conclusions

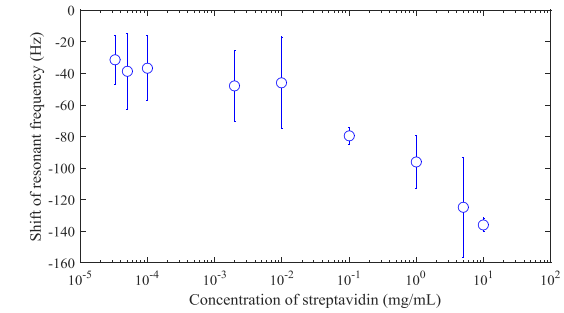
Focusing on amounts of absorbed bubbles and the target molecule on QCM surface, the change in resonant frequency of QCM under interaction between bubbles and target molecule was investigated. As a result, it was experimentally indicated that the amount of absorbed bubbles related to that of target molecule.


## Acknowledgment

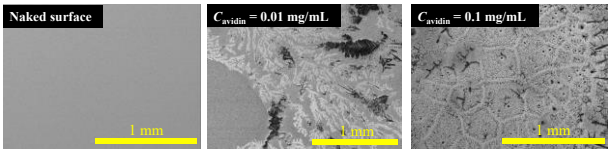
This work was partially supported by JSPS KAKENHI Grant Number 13303779.



kenyoshi1980@chiba-u.jp

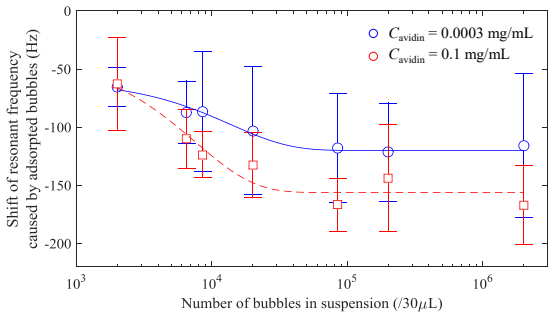




(a)

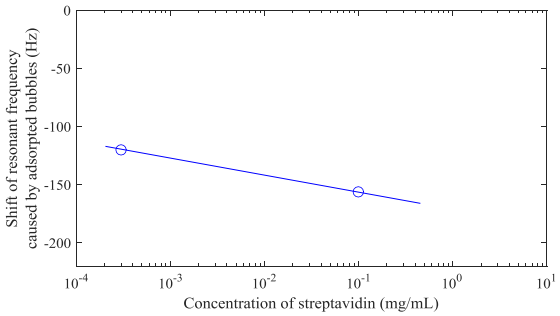



(b)

Fig. 2 Fixation of streptavidin on QCM surface. (a) Resonant frequency shift as function of streptavidin concentration. (b) SEM image on QCM surface. Left, center and right images are obtained in case of control (0 mg/mL), 0.01 mg/mL and 0.1 mg/mL, respectively.




(a)




(b)

Fig. 3 Characteristic of $\Delta f_{\mathrm{b}}$–$N_{bubble}$ and $\Delta f_{\mathrm{b}}$–$C_{avidin}$.

## References


1. T. Muramoto, R. Shimoya, K. Yoshida, and Y. Watanabe: Ultrasound Med.Biol. **40** (2014) 1027.
2. R. Shimoya, K. Yoshida, Y. Yokoi, and Y. Watanabe: Jpn. J. Appl. Phys. **54** (2015) 07HD07.