Sensitive label-free IgG detection using MEMS QCM biosensor with 125-MHz wireless quartz resonator

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1. Introduction

Quartz crystal microbalance (QCM) has been widely applied as biosensors due to its low cost and real-time monitoring capability [1-3]. Especially, wireless-electrodeless QCM technique has been developed [4-5], showing great advantages in biosensing [6-7], diagnosis, study of intermolecular reactions [8] and aggregation of amyloidosis proteins [9]. Unlike conventional QCM, in which, the resonator is coated by thin layers of metal on both sides serving as electrodes, the wireless-electrodeless QCM technique operates in a wireless manner, allowing the use of extremely thin and high frequency QCM. Since the mass sensitivity of QCM is inversely proportional to the square of the resonator thickness, the sensitivity of QCM biosensor can be greatly improved using thinner (higher frequency) and wireless quartz resonator. In our previous study, we succeeded in fabricating MEMS QCM biosensor with naked-embedded wireless quartz resonator [10], where, a long fluid channel was adopted in order to obtain steady flow and stable frequency baseline, resulting in a large sensor size (20 mm in length).

In this study, we demonstrate a new MEMS biosensor chip with wireless QCM coated with gold film on both sides, in which, the length of fluid channel has been reduced by half. Because of the miniaturization, the number of sensor chips fabricated from one silicon wafer can be doubled. The fabricated QCM sensor chip is more compact but without degradation in signal to noise ratio (SNR) or baseline stability. Using the new MEMS QCM biosensor, direct detection of IgG with high sensitivity can be realized without any signal amplification process.

2. Fabrication of MEMS QCM biosensor

Figure 1 illustrates the structure of MEMS QCM biosensor chip. The AT-cut quartz resonator is embedded inside the fluid channel constructed on the inner sides of silicon substrate and the glass substrate. The bottom glass substrate with pre-bonded silicon layer is bonded to the top glass substrate using the anodic bonding method. The resonator is slightly fixed by the micropillars on the bottom and sidewalls of substrate to ensure nearly free vibration. The resonator has an area of 1.8 mm × 1.6 mm and a fundamental resonate frequency of 125 MHz. Layers of 2 nm Cr followed by 8 nm Au are deposited on both sides of the resonator. During measurement, the QCM is excited by antenna, and the resonant frequency is monitored by a vector network analyzer.

The fabricated QCM biosensor chip has several advantages. First, because of wireless operation, the MEMS QCM biosensor can be operated at a high frequency. Second, it's more compact, with an overall size of 10 mm × 5 mm, which makes it suitable for mass production. Moreover, the shorter microfluidic channel makes it possible to perform the bioassay with minimal consumption of analyte, which is important especially in diagnostic application.

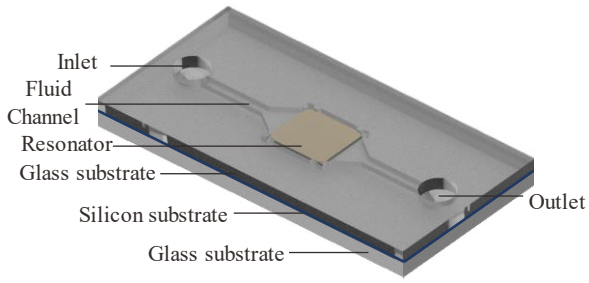

Fig. 1 Structure of newly developed MEMS QCM biosensor.

3. Experiment on IgG detection

The protein A for detecting of IgG is immobilized on the resonator surface as a ligand based on our previous study [7]. We first cleaned the QCM by injecting piranha solution, followed by ultrapure water and absolute ethanol rinsing. Next, we injected 10 mM 10-carboxy-decanthiol in absolute ethanol and stored the sensor chip at 4 ℃ overnight for making the self-assembled monolayer (SAM). Then, a mixture of 100 mM N-hydroxysulfosuccinimide sodium salt (NHS) and 100 mM 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) in ultrapure water was injected to activate the SAM terminals. A 200 μg/mL protein A in phosphate-buffered-saline (PBS) solution was then injected to immobilize protein A on the surfaces. At last, 5 mg/mL bovine

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serum albumin (BSA) in PBS was injected to block the remaining activated terminals.

The QCM chip was then set into the homebuilt sensor cell. A micro-pump was used to flow and circulate the buffer and analyte solutions. The flow rate was about 400 µL/min. Before IgG detection, we first flowed ultrapure water to rinse the QCM and obtain stable frequency baseline. Then we injected analyte containing IgG in ultrapure water. To evaluate the sensitivity and reusability of the sensor chip, we injected IgG solution with different concentrations after flowing 0.1M Glycine-HCl buffer (GHB) solution to remove IgG molecules binding from protein A. The resonate frequency change of QCM during analyte flow was measured to monitor the reaction progress.

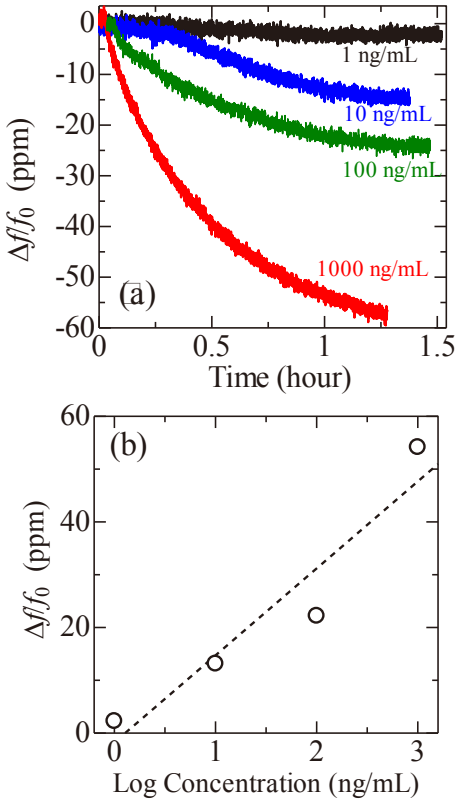

Fig. 2 (a) Frequency responses of QCM in injection of IgG solutions with different concentrations; (b) Decreases in frequency one hour after injection of IgG solution with different concentrations.

4. Results and Discussion

Figure 2(a) shows the resonance frequency change of the QCM in IgG solution flow, reflecting the binding reaction between protein A and IgG molecules. As binding reaction progresses, IgG molecules are captured by protein A, leading to an increase in the adsorption mass on QCM surface, and thus cause the resonance frequency of QCM to decrease. The decrease in resonance frequency increases with the increase of IgG concentration. It's worth noting that the IgG detection with different concentration in **Figure 2(a)** were performed using the same sensor chip after removal of binding IgG by flowing GHB, validating the excellent reusability of the QCM biosensor. We also invested the frequency decline after certain reaction time. **Figure 2(b)** represents the amount of frequency decrease plotted against logarithm of concentration, showing good linearity.

5. Conclusion

We present a compact MEMS QCM biosensor, where a 125 MHz quartz resonator is embedded, and performed the direct detection of IgG. The sensor chip exhibits a high sensitivity; IgG with concentration as low as 1 ng/mL can be detected in a label-free manner. The decrease in the resonate frequency after a certain time has a linear relationship to the logarithm of concentration. Therefore, the fabricated sensor chip, which has advantage of wireless, compact, reusable, easy to mass production and high sensitivity, shows promising application prospect.

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