Study on Protein Detection Using a UHF-Band Wireless QCM Sensor Array Chip

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1. Introduction

The Enzyme-linked immunosorbent assay (ELISA) and surface plasmon resonance (SPR) methods[1,2], which enable multi-channel measurements, are widely used in screening process that require the simultaneous evaluation of many samples, such as in the field of antibody drug discovery. The QCM method can measure biomolecular reactions in real time and evaluate affinity quantitatively; however, it is inferior to the ELISA and SPR regarding high-sensitivity and multi-channel measurements. The sensitivity of the QCM sensor is inversely proportional to the square of the quartz thickness, and therefore increases significantly as the thickness decreases. Commercially available QCM sensors have metallic electrodes on the quartz surface for the excitation and signal detection of the quartz resonator. Therefore, As the quartz thickness becomes thinner, the inertial resistance due to metallic electrode increases relatively, making it difficult to excite the quartz resonator. For these reasons, commercially available QCM sensors generally use quartz resonators with a thickness of 55-330 μm (i.e., with a fundamental resonance frequencies of 5-30 MHz). To improve this issue, the wireless electrodeless (WE) QCM sensor was developed.[3] This sensor was able to use thin quartz resonators with a thickness of less than 10 μm and specifically capture the protein at extremely low concentrations of 1 ng/ml.[4] In this study, the multichannel WE-QCM sensor array chip, which has 100 diaphragm quartz resonators with a thickness of 1.5 μm were integrated on a single chip, was developed by combining WE-QCM sensor technology with silicon (Si) anisotropic etching,

2. Ultra-High Frequency QCM Biosensor Array

Figure 1 shows the appearance of the high frequency WE-QCM biosensor array chip, one diaphragm resonator, and an example of the cross-sectional view of the multilayer substrate. This integrated chip was fabricated using a multilayer substrate in which an AT-cut quartz substrate with a thickness of 1.5 μm and a Si(100) substrate with a thickness of about 500 μm were bonded at room temperature. AT-cut quartz and Si(100) substrates were bonded via a Cr/Au (6 nm/20 nm) adhesion layer to increase bonding strength. No delamination and voids were observed at the bonded interface.

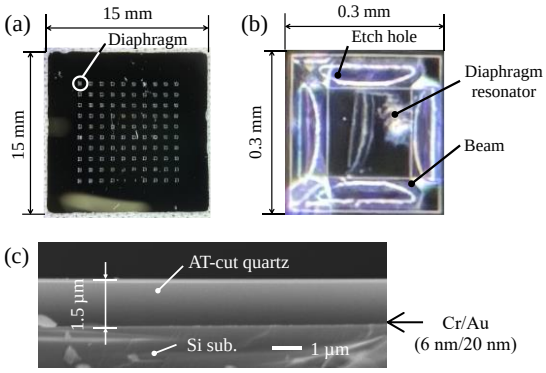

Fig. 1 (a) Photograph of wireless QCM biosensor array chip, (b) enlarged view of diaphragm resonator, (c) cross sectional view of multilayer substrate.

The diaphragm quartz resonator has a unique structure with arc-shaped etch-holes on each of the four sides of the 0.3 mm square membrane. The diaphragm quartz resonator, in which the thin quartz membrane was fixed with four fine beams, was fabricated by anisotropic etching of the Si(100) substrate using these etch-holes. One hundred diaphragm quartz resonators arranged in an array of 10 rows and 10 columns were integrated on a 15 mm square Si(100) substrate. The theoretical fundamental resonance frequency, which is calculated with the sound velocity and thickness of the AT-cut quartz, was about 1.113 GHz.

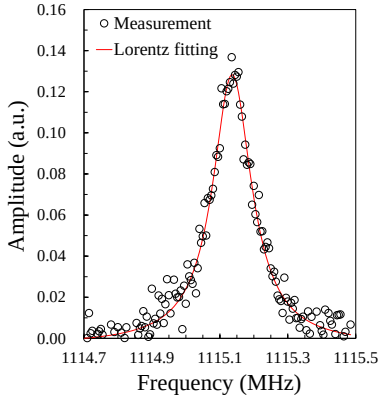

Fig. 2 Resonance spectrum in atmosphere.

The array chip was fabricated using Micro-electro-mechanical systems (MEMS) technology, which applies semiconductor microfabrication. EM

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waves to diaphragm quartz resonator were transmitted and received using two copper (Cu) wire antennas, which were inserted in an aluminum (Al) tube for EM waves shielding, for each resonator to measure the fundamental resonance frequency. **Figure 2** shows a resonance spectrum in the atmosphere. The fundamental resonance frequency was about 1.115 GHz, and the quality factor (Q-factor) was about 13,000.

3. Protein Capture Process Using a Sensor Array

Figure 3 shows the process of specifically capturing the target protein by utilizing the nonspecific adsorption of proteins to the quartz surface. The array chip was immersed in the piranha solution, which is a mixture of H_2SO_4:H_2O_2 (3:1) to remove organic contaminations adsorbed on the diaphragm surface, and then rinsed with distilled water to activate surface. The array chip was placed in a petri dish filled with the silica gel and stored in a desiccator to thoroughly remove moisture from both inside the cavity and the surface of the chip. Subsequently, the resonance frequency in atmosphere was measured for each diaphragm and used as the baseline. In this bioassay, ultrapure water (UPW) buffer was used to dilute each solution.

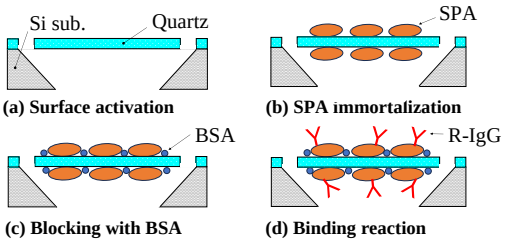

Fig. 3 Specific binding of R-IgG via SPA.

Fig. 4 Frequency changes due to mass loading.

The array chip was immersed in a 0.1 mg/ml solution of staphylococcal protein A (SPA) to nonspecifically adsorb SPA molecules on the diaphragm surface. The array chip was then immersed in a 10 mg/ml solution of bovine serum albumin (BSA) to block the quartz surface. After rinsing with UPW, the moisture was thoroughly removed again, and the resonance frequency was measured for the second time. Next, the array chip was immersed in a 10 μg/ml solution of rabbit immunoglobulin G (R-IgG) to specifically bind R-IgG molecules via nonspecifically adsorbed SPA, and then it was rinsed with UPW. Afterwords, the moisture was thoroughly removed, and the resonance frequency was measured for the third time. **Figure 4** shows the resonance frequency changes obtained for each step.

4. Results and Discussion

The high frequency QCM biosensor array chip was fabricated from a multilayer substrate, which was made of AT-cut quartz and Si substrates bonded at room temperature, by utilizing MEMS technologies. If a diaphragm is formed using a multilayer substrate fabricated through a bonding process involving heating, buckling is inevitable due to the difference in internal stress caused by the difference in thermal expansion coefficients. For this issue, we successfully fabricated the array chip, which had diaphragm resonators without buckling with good resonance spectra, by using a multilayer substrate bonded at room temperature. Using the fabricated array chip, it was performed to capture target proteins specifically via proteins adsorbed nonspecifically on diaphragm resonators. When SPA and BSA molecules were adsorbed on the surface of a diaphragm quartz resonator nonspecifically, a frequency change of about 145 kHz was obtained. This frequency change indicated that a total of about 0.030 ng of SPA and BSA molecules were adsorbed nonspecifically. In addition, when R-IgG molecules were specifically captured via SPA molecules, a frequency change of about 130 kHz was obtained. This frequency change indicated that a total of about 0.028 ng of R-IgG molecules was bonded specifically. These experimental results revealed that the WE-QCM sensor array chip developed in this study would be useful as a biosensor.

5. Conclusion

UHF-band WE-QCM sensor array chip was fabricated by MEMS techniques using a multilayer substrate bonded AT-cut quartz and Si substrates at room temperature. We succeeded in measuring frequency changes due to the specific binding between R-IgG and SPA molecules using the WE-QCM sensor array chip.

References

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