

Proceedings

# Fabricating a Highly Sensitive QCM Sensor Using AAO Nanoholes and Its Application for Biosensing <sup>†</sup>

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**Abstract:** A nanostructure composed of Anodic Aluminum Oxide (AAO) was obtained on an electrode of a quartz crystal microbalance (QCM) chip by anodizing Al thin film in an oxalic acid solution. The effective surface area was expanded by these nanostructures. Several morphologies were observed under various anodic conditions by using scanning electron microscopy (SEM). We demonstrated that a QCM chip with the AAO was effective in biosensing because of its large surface area. The frequency shift corresponding to an antigen-antibody reaction improved on the nanostructured electrode compared with a flat surface electrode.

**Keywords:** biosensing; QCM; anodic aluminum oxide; nanostructure

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## 1. Introduction

In the recent years, many biosensing systems, for examples, surface plasmon resonance (SPR) and enzyme-linked immune sorbent assay (ELISA) are widely used. Both technics can detect analyte with high sensitivity. However, they have disadvantages. In the SPR, it's low throughput technic and it needs complex optical equipment [1]. In the ELISA, it's time consuming method. Additionally, it needs professional skill and labeling material [2]. Therefore, some conditions are required for biosensing as follows; low cost equipment, rapid analysis, real time measurement, high sensitivity and convenient protocol. If these requires are achieved, we can perform biosensing system easily. In this study, we focused on a quartz crystal microbalance (QCM) method by following reasons. QCM method is one of promising approaches to detect gas molecules and bio materials. In addition, it has advantages in real time measurement, high sensitivity, and portability. However, the method has poor sensitivity to low molecular weight materials (<1 kDa) because of its measuring principle, mass change. Theoretical mass change by absorption of analyte on the electrode over the quartz is estimated by using Sauerbrey equation [3]. In fact, the magnitude of the frequency shift is proportional to the mass change. For an example, the mass change is calculated as 1.07 ng with the frequency shift of 1 Hz on the fundamental frequency of 9 MHz. On the analysis of 1 kDa material, the frequency shift of 1 Hz is about  $10^{-12}$  mol. Its concentration is too low to detect. Some groups introduced to fabricate the nanostructures on the electrode of QCM chip to improve the sensitivity for the detection of the low molecular weight materials. For example, a nanostructured IrO<sub>2</sub> was manufactured on the gold electrode by metal organic chemical vapor deposition (MOCVD) [4]. The sensor could detect volatile organic compounds (VOC). However, it was difficult to control the nanostructures of IrO<sub>2</sub>, and the material was hazardous. The nanostructured QCM dynamically monitored antimicrobial resistance using magnesium zinc oxide [5]. As the results, the surface area was enlarged by nanostructures, and the amount of analyte binding on the surface was greatly improved. However, the experimental protocol wasn't convenience because the protocol labeled the bacteria to antimicrobial on the electrode. Here, we focused on nano porous structure of anodic aluminum oxide (AAO) to detect the analyte with highly sensitivity. AAO is obtained by only

anodizing under a suitable potential in electrolytic solution [6]. Then, self-organized nanoholes having a triangle lattice is formed in a downward direction with high aspect ratio. And the aspect ratio is controllable by anodic condition, for example type of electrolyte solution, applying potential, anodization time and solution temperature [7]. In this study, we attempted to fabricate AAO nanostructure on the electrode of quartz crystal, and the surface area was estimated. The sensitivity on the antigen-antibody interaction was evaluated by using Anti-Mouse IgG and Mouse IgG in the QCM measurement.

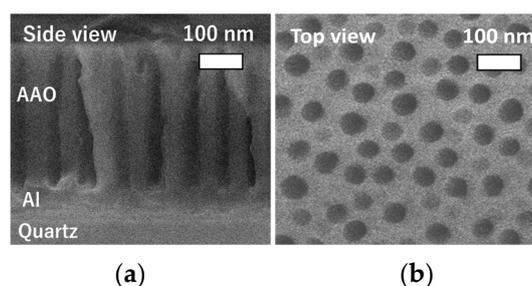
## 2. Materials and Methods

All the reagents used here were purchased from Wako Pure Chemicals Co. Ltd (Osaka, Japan). Firstly, titanium (Ti, 20 nm) and aluminum (Al, 500 nm) was sputtered on a quartz crystal in this order which fundamental frequency was 9 MHz. Secondly, Al thin film was anodized under the applied voltage of 40 V in 0.3 M oxalic acid for 1 to 4 min. Finally, a diameter of nanohole was widened in 5 wt % phosphoric acid. Then, we obtained QCM chip with AAO nanostructure on the surface of the quartz. The morphology of AAO nanostructure was observed by scanning electron microscopy (SEM; JEOL JSM-7500F). A preparation for measuring antigen-antibody interaction was explained as follows:

Self-assembled monolayer (SAM) consist of APTES (3-aminopropyltriethoxysilane) was formed on the surface of the AAO nanostructure. Anti-Mouse IgG (100 µg/mL) and Bovine serum albumin (BSA; 0.01%) were immobilized on the surface of AAO nanostructure through SAM for 10 min, in this order. Then, the QCM chip was set on a measurement cell. Finally, Mouse IgG (10 µg/mL) was injected in the measurement cell and monitored a frequency shift of the QCM.

## 3. Result and Discussion

Figure 1a, b show cross and surface sectional SEM images of AAO nanostructure on the quartz crystal. The AAO was fabricated under the voltage of 40 V for 4 min. At first, the aluminum thin film was sputtered 500 nm. While, it decreased to be about 100 nm, and a thickness of the obtained AAO nanostructure was 450 nm after anodization. The diameter of nanohole was increased to be 50–60 nm by etching the AAO using phosphoric acid. The estimated hole density was 100 piece per square µm.



**Figure 1.** Cross sectional (a) and surface (b) SEM images of AAO nanostructure on the quartz crystal. Applied voltage was set as 40 V for 4 min in the oxalic acid solution.

Table 1 shows the ratio of surface area compared with non-anodic electrode, flat surface. The values were calculated using the Equation (1) as follows. The surface area of the nanostructure was estimated using the length, radius and density of the nanohole, and was ranged from 3.1 to 9.3.

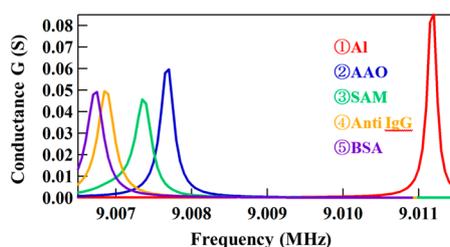
$$\text{Ratio of surface area} = \frac{\text{Surface of AAO nanostructure}}{\text{non - anodic electrode}} \quad (1)$$

Figure 2 shows conductance measurement depending on the preparation process of QCM measurement. The frequency shift decreased for each process in order. The results indicated the QCM chip was affected by each mass changes. Therefore, Anti-Mouse IgG and BSA were immobilized on the surface of AAO nanostructure normally through amino groups on the SAM. Figure 3 shows a frequency shift of the QCM measurement in the antigen-antibody interaction depending on the ratio of the surface area. The obtained frequency shifts were approximately 50, 100, 200, 500 Hz on each

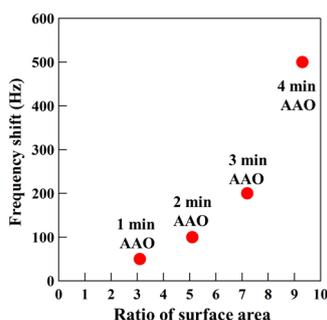
AAO nanostructured QCM chip. The magnitude of frequency shift increased with the ratio of the surface area. The result indicates that sensitivity of the AAO nanostructured QCM chip was improved by the nanostructure and the obtained frequency shift was 10 times larger than that on the flat surface.

**Table 1.** Estimated ratio of surface area.

Anodic time (min)	0	1	2	3	4
Ratio of surface area	1.0	3.1	5.1	7.2	9.3



**Figure 2.** The conductance measurement of the AAO nanostructured QCM chip under applied voltage of 40 V for 4 min for each preparation process.



**Figure 3.** Frequency shift of the antigen-antibody interaction using the QCM with AAO nanostructure. Concentration of antigen (mouse IgG) was 10  $\mu\text{g/mL}$  in the all experiments.

#### 4. Conclusions

An aluminum was sputtered on the electrode of QCM chip, and it was anodized in the oxalic acid solution. Then, it succeeded to fabricate AAO nanostructured QCM chip having high aspect nanoholes array. The diameter of nanohole was ranged from 50 to 60 nm. The density of the nanoholes was approximately 100 pieces/ $\mu\text{m}^2$ . The surface area of AAO nanostructure by applied voltage of 40 V for 4 min was enlarged approximately 9.3 times comparing with non-anodic electrode QCM chip. In the QCM measurement with antigen-antibody interaction, the obtained frequency shift was 500 Hz as the maximum value. Comparing AAO nanostructured and non-anodic QCM chip, the sensitivity was improved by large surface area of AAO.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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