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Flow-Injection Wireless-Electrodeless QCM System Combined with Total Internal Reflection Fluorescence Microscopy

全反射蛍光顕微鏡とフローインジェクション型無線無電極 QCM システムの融合

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1. Introduction

The quartz crystal microbalance (QCM) biosensor is a mass-detectable sensor which can detect mass change on its surface through the change in the resonance frequency of the oscillator. When the receptor modified quartz-crystal resonator captures the receptor-specific molecules, the effective mass of the resonator increases. Then, the resonance frequency of the resonator decreases. Hence, we can measure interactions between biomolecules quantitatively and in real-time.

However, conventional QCM biosensor has the disadvantage; the sensitivity is lower than that of other biosensors such as SPR and ELIZA. To measure interactions between biomolecules precisely, a higher-sensitive QCM is necessary. It can be achieved by making quartz crystal oscillator thinner because the sensitivity of QCM is inversely proportional to the square of its thickness. However, making QCM thinner causes further problem. Conventional QCM has gold layers (as electrodes) and wires on the surface of the oscillator to generate and detect oscillation by the electrical means. These electrodes and wires prevent oscillation of quartz crystal as its thickness decreases. Thus, removing electrodes and wires is essential to improve the sensitivity of QCM. We have thus developed Wireless-Electrodeless QCM (WE-QCM) biosensor and achieved higher sensitivity [1].

Here, we propose the flow-injection wireless-electrodeless QCM combined with total internal reflection fluorescence (TIRF) microscopy as a new research device for life science studies. TIRF achieves high spatial resolution owing to the evanescent-light field, which is generated by the total internal reflection of the excitation light on the cover glass surface as the excitation source. This combination is possible only with our WE-QCM since conventional QCM and SPR have electrodes on its surfaces.

By performing QCM measurement along with the TIRF observation simultaneously, the TIRF-QCM sensor will be the only tool which can evaluate the location and quantity interacted biomolecules simultaneously. In this study, we develop the flow-injection TIRF-QCM for the first time and examine the aggregation reaction of Aβ peptide which is closely related to Alzheimer's disease (AD).

2. TIRF-QCM

In vivo, the first step in the aggregation process of Aβ peptide is deposition of amyloid nuclei on the surface of nerve cell. Then, Aβ monomers aggregate on them to form fibrils and neurotoxic oligomers [2]. In order to duplicate this condition, we immobilized amyloid nuclei on the surface of the quartz-crystal oscillator, and flowed monomers.

Figure 1 shows originally developed TIRF-QCM sensor cell in order to flow monomer solutions. Excitation light must satisfy the total reflection condition on upper surface of the oscillator where Aβ peptides aggregate. Hence, oscillator is placed on cover glass and held lightly by silicon rubber sheet, where the flow path is composed. Two enamel wires are used as transmitting and receiving antennas in order to generate and detect oscillation through electromagnetic wave, respectively. This sensor cell enables us to measure aggregation process by WE-QCM and to observe fibril elongation by TIRF simultaneously.

For observing Aβ peptides aggregation with TIRF, we used Thioflavin T (ThT) as fluorescent molecule, which has been adopted for evaluating formation of protofibrils and their elongation. The benzothiol-dye ThT specifically binds to β-sheets constructing amyloid fibrils and produces enhanced light emission.

3. Experimental Procedure

First, a naked quartz crystal oscillator was cleaned in the piranha solution (98% H_2SO_4: 33% H_2O_2 = 3:7), and then rinsed with ultrapure water. Aβ peptides were dissolved in a dimethyl sulfoxide (DMSO) solution and diluted by buffer solution (containing 100 mM NaCl) to the concentration of 50 μM. The nuclei were formed by stirring the peptide solution at 1200 rpm. The nuclei solution is mixed with ThT to TIRF observation. Final concentrations of the nuclei and ThT were 10 μM and 20 μM, respectively. In order to immobilize the nuclei on the surface of oscillator in a non-specific manner, the oscillator was immersed in the solution containing the nuclei for 1 h at 4 °C.

The sensor cell was incorporated in the flow-injection system. The monomer solution was mixed with ThT, and final concentrations of monomers and ThT were 30-40 μM and 20 μM, respectively. Flow rate of the monomer solution was 200 μl/min.

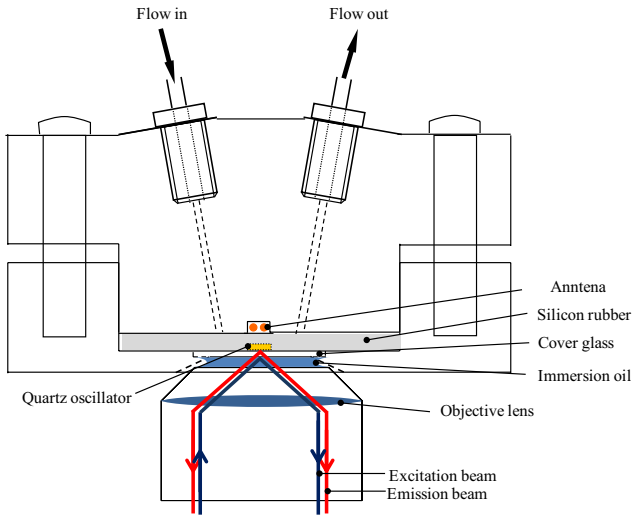

Fig. 1 Orignally developed TIRF-QCM sensor cell.

3. Results

Figure 2 shows the example of aggregated number of moles based on change in resonance frequency during the monomer flow. We have succeeded in monitoring the aggregation process of Aβ peptides with WE-QCM. The decrease in resonance frequency indicates that Aβ monomers were deposited on the nuclei. Florescence images of Aβ aggregates during the aggregation experiment were obtained by TIRF as shown **Fig. 3**.

4. Conclusion

We have developed flow-injection wireless-electrodeless QCM system combined with total internal reflection fluorescene microscopy. This system enables us to evaluate quantitatively the aggregation of Aβ peptides by WE-QCM and observe the fibril formation by TIRF at the same time for the first time.

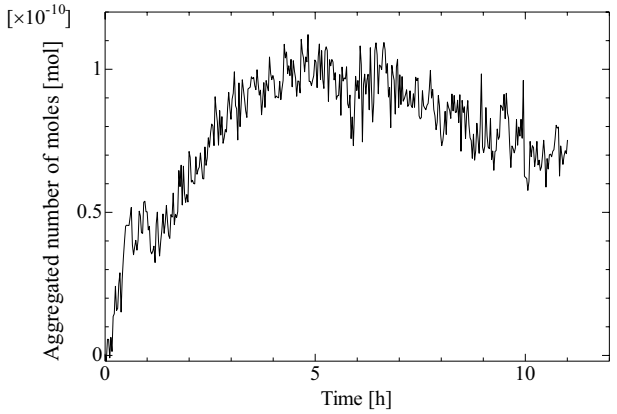

Fig. 2 Aggregated number of moles based on change in resonance frequency during the aggregation experiment

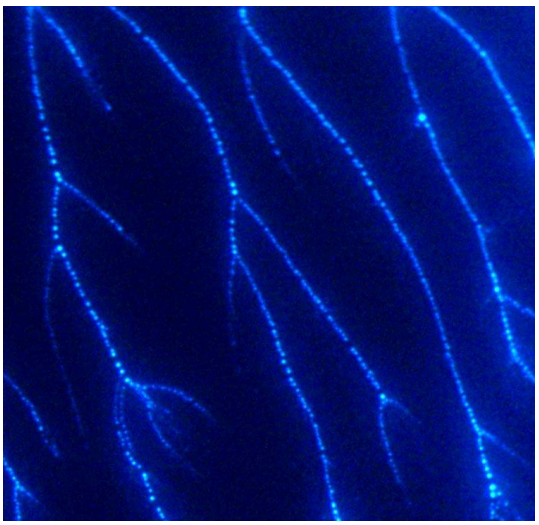

Fig. 3 TIRF images of Aβ aggregates during the aggregation experiment.

5. References

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