Development of a simultaneous monitoring system for viscoelastic properties and morphological changes of cultured cells using wireless and electrodeless QCM

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1. Introduction

In recent years, many studies have demonstrated that cellular functions are closely related to the viscoelastic properties of cells[1,2]. For example, viscoelasticity of the extracellular matrix can regulate the differentiation of mesenchymal stem cells (MSCs)[3,4]. Therefore, it has become significantly important to monitor changes in the viscoelastic properties of living cells for a deeper understanding of the relationship between cellular functions and their mechanical properties. Various techniques have been utilized to measure cellular viscoelasticity, including atomic force microscopy[5] and micropipette aspiration[6]. Although these existing techniques provide viscoelasticity of cells, they require physical contact with cells, causing the cell damage and making long-term monitoring difficult. Therefore, a non-invasive monitoring system that can monitor the viscoelastic properties of living cells over a long period of time is needed in the field of mechanobiology.

Quartz-crystal-microbalance (QCM) is a non-invasive technique for evaluating change in the cell viscoelasticity. QCM is usually used for detecting target molecules captured on the QCM surface by corresponding ligands immobilized there through the resonance-frequency change of the quartz resonator caused by the mass loading effect.[7] The resonance frequency also reflects the viscoelastic properties of the adsorbed layer[8]. QCM is thus capable of providing us with information on the viscosity change of cells in culture, without causing cell damage. However, previous studies with conventional QCM[9] needed the metallic electrodes on both surfaces for exciting the vibration and failed to monitor the morphological change of the cells at the same time because of the opaqueness of the electrodes. The morphological change has to be monitored along with the QCM measurement, because we cannot evaluate the viscoelasticity of cells only from the frequency change when the cell density varies. For example, if an increase in the resonance frequency is observed, it is not possible to determine whether it is due to an increase in the stiffness[10] or a decrease in the mass caused by cells' detachment from the QCM surface.

In this study, we originally develop an advanced measurement system for the simultaneous monitoring of viscoelastic properties and morphological changes of living cells. We use the wireless and electrodeless QCM[11], which allows us to combine the QCM measurement with a microscopic observation through the transparent quartz crystal so that we can investigate the relationship between the viscoelasticity and morphological change.

We adopt this system to monitor the activation process of group 2 innate lymphoid cells (ILC2s)[12]. ILC2s play an important role in helminth infections and allergic diseases[13,14]. They change their size upon activation suggesting that their mechanical properties are also affected by the activation process. However, there are no reports on the mechanical properties of ILC2s during their activation process.

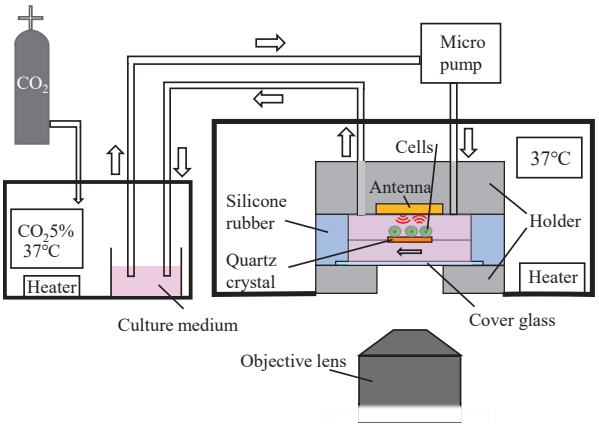

Fig. 1 Schematic of the developed system.

2. Experiment

We used a blank AT-cut quartz crystal with a rectangular parallelepiped area of 1.8×1.6 mm^2, and thickness of 26 μm, showing the fundamental through-thickness shear-mode resonance frequency of 64.5 MHz. **Figure 1** shows the schematic of the developed system. This allows microscopic observation of the quartz crystal surface through the holder under the cover glass, simultaneously performing the viscoelasticity monitoring by QCM. After the quartz crystal and cover glass were washed with a piranha solution, the quartz crystal was sandwiched by silicon-rubber sheets and placed under the antenna that excites and detects the

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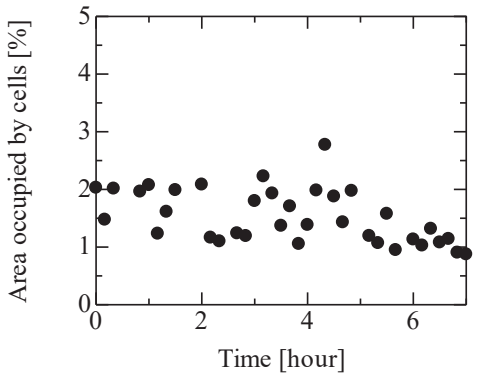

Fig. 2 Percentage of area occupied by cells on the quartz crystal.

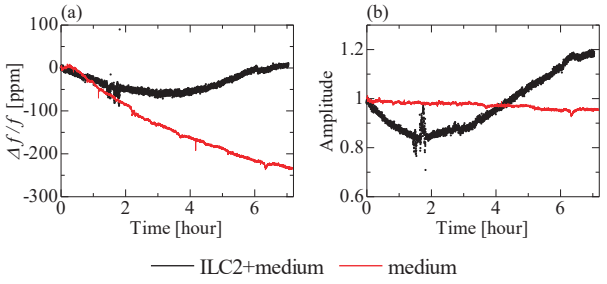

Fig. 3 (a)Resonance frequency change and (b) vibrational amplitude change of the quartz crystal with ILC2s (black). The red lines show the control experiments when only the culture medium was injected without cells.

vibration of the quartz crystal contactlessly via the electromagnetic waves. The QCM holder was fixed on the optical-microscope stage for the dark-field observation. The ILC2s suspension medium was injected through the inlet into the microchannel. We then started measurements of the resonance frequency and the morphology of the cells on the quartz crystal.

3. Results and Discussion

With this experimental system, the viscoelasticity monitoring by QCM and microscopic observation has been conducted simultaneously on living cells for more than six hours. **Figure 2** shows the change in the occupied area by cells on the quartz crystal, calculated from the microscopic images. It is nearly unchanged up to ~5 h, after that it appears to decrease slightly. **Figure 3** shows examples of monitoring of the resonance frequency change and the vibrational amplitude change of the quartz crystal. After the injection of the cells, both the resonance frequency and vibrational amplitude continue to decrease for ~2 h. The decrease of the resonance frequency indicates the increased adhesion strength to the substrate so that the inertia resistance due to cells is enhanced. This is supported by the amplitude decrease; the vibrational amplitude should decrease with the enhancement of the inertia resistance. Very interestingly, they increase after ~3 h, exceeding their initial values at 6 h. Considering that the cell density remains unchanged up to 6 h, the increase in the resonance frequency indicates the stiffness increase[10] and the vibrational amplitude increase suggests a decrease in cells' overall viscosity. These behaviors are quite different from the control experiment in which only the culture medium was injected (red marks in Fig. 3); both the resonance frequency and vibrational amplitude continue to decrease in this case, which can be attributed to the nonspecific absorption of various proteins in the culture medium.

4. Conclusion

We originally developed a simultaneous monitoring system for viscoelastic properties and morphological changes of living cells. We then apply it to ILC2s and successfully observed characteristic viscoelastic behavior during culturing. We will further study the relationship between viscoelasticity change and morphological change during the ILC2 activation.

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