Development of a simultaneous measurement system of wireless-electrodeless QCM and optical microscopy for monitoring changes in mechanical properties of live cells in culture

Motoyuki Hamana[1‡], Natsumi Fujiwara[1], and Hirotsugu Ogi[1*]
([1]Grad. Sch. Eng., Osaka Univ.)

1. Introduction

In recent years, many studies have shown that cellular functions are closely linked to the mechanical properties of living cells[1,2]. For example, mesenchymal stem cells change their differentiation by regulating their mechanical properties in response to the viscoelasticity of the extracellular matrix[3,4]. Therefore, it has become significantly important to monitor changes in the mechanical properties of living cells to understand more deeply the relationship between cellular functions and their mechanical properties. Various techniques, such as atomic force microscopy[5] and micropipette aspiration[6] have been widely used to measure the mechanical properties of cells. However, they can cause serious damage to cells because of physical contact with cells and preventing long-term monitoring. Therefore, it is necessary to develop a non-invasive measurement system for monitoring the mechanical properties of living cells over a long period of time.

In this study, we apply quartz-crystal-microbalance (QCM) to monitor the mechanical properties of living cells to solve the problem mentioned above. QCM is a non-invasive technique and realize monitoring changes in the cell viscoelasticity. Generally, QCM is used to detect target molecules captured on quartz crystal surface by corresponding ligands, based on resonance frequency shifts caused by mass loading[7]. The resonance frequency also reflects the viscoelastic properties of the absorbed layer[8]. Consequently, QCM can provide us with insights into the viscoelasticity changes of cells in culture without causing cell damage. However, conventional QCM[9] setups require metallic electrodes on both surfaces for exciting and detecting the resonator vibration, which made simultaneous microscopic observation difficult due to opaqueness of the electrodes. Monitoring morphological changes is essential, as it is difficult to evaluate changes in mechanical properties solely from frequency changes when cell density varies. For example, an increase in the resonance frequency could be attributed either to increased stiffness[10] or decreased mass resulting from cell detachment from the quartz crystal surface. Therefore, we here develop an advanced measurement system for simultaneous QCM and microscope observation of living cells. We apply the wireless and electrodeless QCM[11], which allows us to combine the QCM measurement with microscopic observation through the transparent quartz crystal so that we can investigate the relationship between the mechanical properties and morphological changes of living cells.

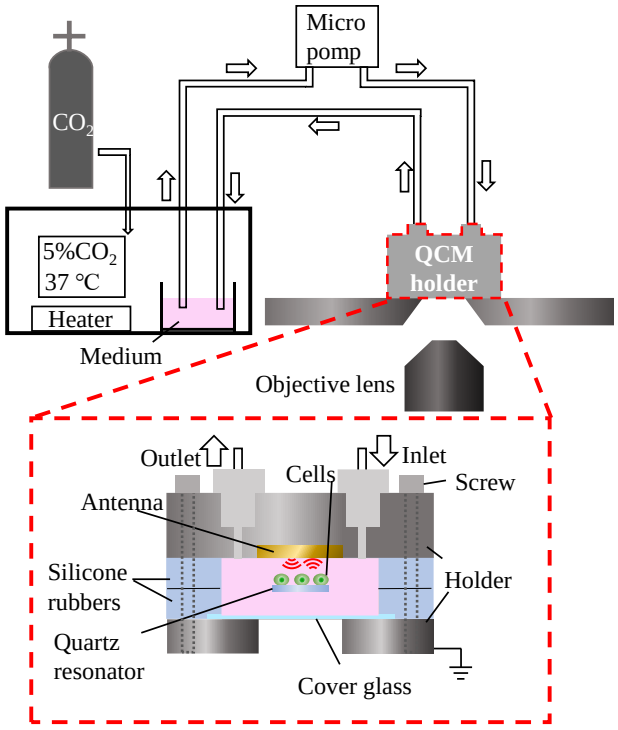

Fig. 1 Experimental setup for QCM measurement and optical microscopic observation of cells in culture

2. Experiment

Figure 1 shows the schematic of the developed system. In this system, we use a blank AT-cut quartz crystal with a rectangular parallelepiped area of 2.5×1.7 mm^2, and thickness of 26 μm, showing the fundamental through thickness shear-mode resonance frequency of 64.5 MHz. This

E-mail: ‡hamana@qm.prec.eng.osaka-u.ac.jp
*ogi@prec.eng.osaka-u.ac.jp

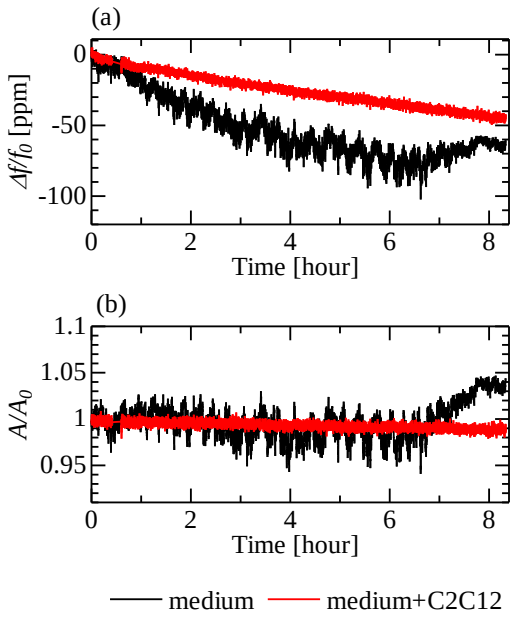

Fig. 2 (a)Resonance-frequency change and (b)vibrational-amplitude change of the quartz crystal with C2C12(red). The black lines show the control experiments when only the culture medium was flawed without cells.

setup enables simultaneous optical microscopic observation of the quartz crystal surface through the holder under the cover glass while monitoring mechanical properties changes by QCM. The quartz crystal is sandwiched by silicone-rubber sheets and placed under the antenna, which excites and detects its vibration without contact via the electromagnetic waves. To promote cell adhesion to quartz crystal, the quartz crystal is coated with fibronectin solution, which is a cell adhesion molecule, and then cells are seeded onto the quartz crystal resonator.

In this study, we used myoblasts C2C12 for the measurement. The experiment was conducted at 37 ℃ and 5% CO_2 concentration.

3. Results and Discussion

With the developed system, QCM measurement and microscopic observation has been conducted simultaneously on living cells for more than 8 hours. **Figure 2** shows monitoring of the resonance frequency change and the vibrational change of the quartz crystal. The resonance frequency continues to decrease, and the vibrational amplitude does not change significantly for about 8 hours. The resonance frequency behavior is different from the control experiment in which only the culture medium was flawed (black line of Fig. 2). In the experiment with only the culture medium, the resonance frequency decreases by about 100ppm, which can be attributed to the nonspecific absorption of various proteins in the culture medium to the

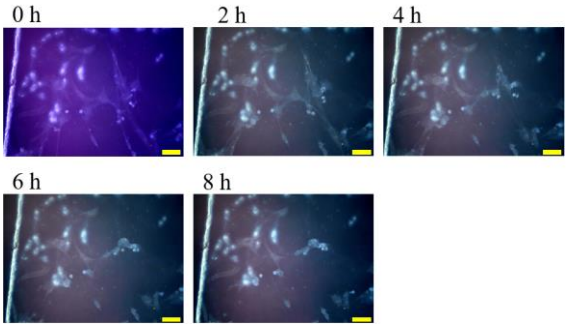

Fig. 3 Optical microscopy images of C2C12 on the quartz crystal. The scale bars indicate 50 μm.

quartz crystal. In contrast, the decrease in resonance frequency observed in the cell measurement is about 50ppm, which is less than that observed with the culture medium one. That suggests the resonance frequency increases due to cellular change. The increase of the resonance frequency indicates that the decreased cell adhesion strength to the quartz crystal. This is supported by the microscope images shown in **Fig. 3**. The images indicate that the cell adhesion area to the quartz crystal decrease over time. Consequently, the decrease in the absorbed mass by cells on the substrate lead to the increase in the resonance frequency compared to the experiment with only culture medium.

4. Conclusion

We originally developed a simultaneous measurement system of wireless and electrodeless QCM and optical microscopy. We then apply it to C2C12 cells and monitored living cells for more than 8 hours. We will further study the relationship between mechanical properties and cellular function changes during culturing. Our system will play an important role in the field of mechanobiology.

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