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Monitoring of morphology and physical properties of cultured cells using a micro camera and a quartz crystal with transparent indium tin oxide electrodes after injections of glutaraldehyde and trypsin

Hyen-Wook Kang, Kazumi Ida, Yuji Yamamoto[1]*, Hiroshi Muramatsu*[*]

School of Bioscience and Biotechnology, Tokyo University of Technology, 1404-1 Katakura, Hachoji, Tokyo 192-0982, Japan

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ABSTRACT

For investigating the effects of chemical stimulation to cultured cells, we have developed a quartz crystal sensor system with a micro charge-coupled device (CCD) camera that enables microphotograph imaging simultaneously with quartz crystal measurement. Human hepatoma cell line (HepG2) cells were cultured on the quartz crystal through a collagen film. The electrode of the quartz crystal was made of indium tin oxide (ITO) transparent electrodes that enable to obtain a transparent mode photograph. Glutaraldehyde and trypsin were injected to the chamber of the cells, respectively. The response of the quartz crystal was monitored and microphotographs were recorded, and the resonance frequency and resonance resistance were analyzed with an *F–R* diagram that plotted the resonance frequency and resonance resistance. In the case of the glutaraldehyde injection, the cells responded in two steps that included the fast response of the cross-linking reaction and the successive internal change in the cells. In the case of the trypsin injection, the responses included two processes. In the first step, cell adhesion factors were cleaved and the cell structure became round, and in the next step, the cells were deposited on the quartz crystal surface and the surface of the cells was directly in contact with the quartz crystal surface.

1. Introduction

For cellular analysis, cultured cells are widely used to simulate biological organisms. In these analyses, a study of morphology is important for understanding the effect of chemical stimulations. A thin lipid membrane is formed on the surface of a cell, and a number of proteins are dispersed in the lipid membrane. Some of them work as structural links between a cytoskeleton and an extracellular matrix (ECM). Thus, the morphology and physical properties reflect a state of the cytoskeleton and activity of cells.

The morphology of the cell has been observed by various methods. The optical microscope can easily observe the shape of a cell. The phase contrast microscope presents the shape of a transparent sample neatly. The cytological staining method provides various applications, such as the distinction of life and death, marking specific targets. The scanning electron microscope and the scanning force microscope are used for more accurate observations. However, estimating the physical properties of the cell by these methods is difficult.

One method to evaluate the physical properties of cells is the quartz crystal microbalance (QCM). The QCM has been

[*] *Corresponding author*. Tel.: +81 42 637 2369; fax: +81 42 637 2369.
E-mail address: muramatu@bs.teu.ac.jp (H. Muramatsu).
[1] Current address: Seiko EG&G, Takatsukashinden, Matsudo, Chiba 270-2222, Japan.
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used as sensors for many purposes, because its resonance frequency changes by mass loading onto the surface of electrodes in nanogram order. The QCM can monitor the mass change in real time as well as quantitatively, which are the significant advantages of the QCM. The mass sensitivity enables the QCM to be used as an accurate sensor.

After the report of Nomura et al., the QCM has been used in a liquid phase [1,2], and also been used as a biosensor [3,4]. Using the QCM in liquid phase, the quartz oscillation is affected by its density and viscosity of the liquid [5].

Dependence of resonance properties of a quartz crystal on liquid viscosity and density has been reported [6–9]. The resonance resistance of the quartz crystal has shown a linear relation to the square root of liquid viscosity and density [8]. Applying these characteristics, the quartz crystal has been used in monitoring the viscosity of liquids and in investigating the viscoelasticity of thin films [10–21]. Similar parameters of the resonance resistance such as energy dissipation [22], the maximal oscillation amplitude [23], etc., have also been proposed.

Comparing the resonance frequency and the resonance resistance is very useful for analyzing the viscoelastic properties of the quartz crystal resonator. Using the resonance frequency and the resonance resistance, the *F*–*R* diagram can be graphed with the resonance frequency on the *X*-axis and the resonance resistance on the *Y*-axis, which is useful for evaluating the mass loading and viscoelastic properties at the same time [11]. The *F*–*R* diagram has been used to evaluate chemical, physical, and biological reactions on the surface [10–20].

A number of studies have been reported on cultured cells using the QCM [18,22–28]. These studies suggested that the mass is not the only factor for the resonance frequency change in the cell attachment process to the quartz crystal [24,26]. Several groups have considered the rheological change on the surface of the quartz crystal in addition to the mass change. For example, the resonance resistance of the quartz crystal was taken into account for analyzing the rheological properties [18,19,28]. Other parameters of the quartz crystal, such as the energy dissipation, the transient decay time, and the maximal oscillation amplitude, were also considered for understanding the rheology [22,23,25,27]. These reports have indicated that the rheological change on the surface of the quartz crystal is effective for understanding cellular process.

A common measurement result is found from these reports. The region that the resonance frequency increased is present in spite of mass loading by cell attachment. Attached cells establish focal contacts with ECM, while the rest surface is not in contact with ECM [29–31]. Thus, Marxer et al. suggested that the mechanical properties of each cell also affect the QCM response [23,27].

In these studies, the importance of the cell concentration was verified to obtain a meaningful resonance frequency change of the QCM. Marx et al. have shown the dependence of the cell concentration on the resonance frequency and the resonance resistance of the quartz crystal by counting the cells at the end of the experiments [19]. To understand the changes in the cultured cells, not only do the parameters of the resonance frequency and resonance resistance change but the microscopic observation is also valuable. In this sense, several groups have performed the QCM measurements and the microscopic measurements simultaneously [18,19,23,28].

For the simultaneous measurement of the QCM and the microscopy, we have constructed a QCM system with a small microscope that can function in a CO_2 incubator. Using the instrument, two chemicals, glutaraldehyde and trypsin, were used to investigate their effects on the cultured cells. As the two chemicals have completely different functions, comparing two different reactions are expected to provide changes on the physical properties of cells reflecting the state of the cells, such as the cytoskeleton, cell attachment properties, and cell activities.

2. Materials and methods

2.1. *Experimental setup of QCM*

Fig. 1 shows the QCM experiment setup. The change in the resonance frequency and the resonance resistance were measured with the QCA922 (Seiko EG&G, Japan). The measurement chamber consists of a quartz crystal, a set of sensor holders that fixes the quartz crystal, and a micro CCD camera (Plumnet, Japan). The base resonance frequency of the quartz crystal was 9 MHz (AT-cut, 7.9 mm × 7.9 mm). Indium tin oxide (ITO) was sputtered onto both surfaces of the quartz crystal as electrodes, which enabled to observe the electrode surface in transmission mode with the micro CCD camera. The white LED over the dish was turned on during observation with the micro CCD camera. A couple of o-rings prevented leakage of the solution. The measurement chamber was autoclaved and put into the incubator (37 °C, 5% CO_2, humid). The QCA922

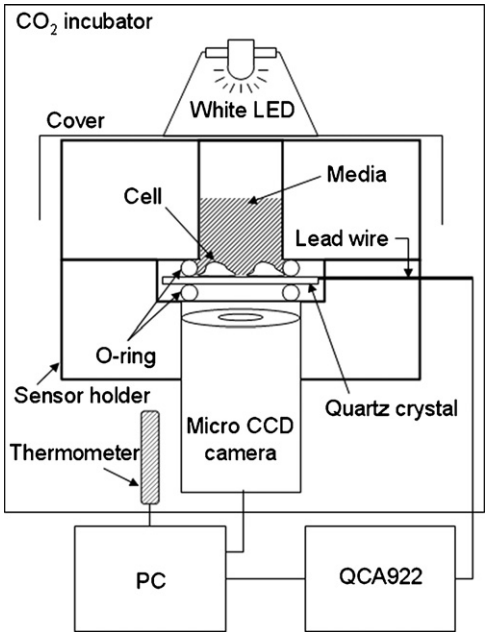

Fig. 1 – Schematic illustration of the QCM experimental setup. ITO was sputtered onto both surfaces of the quartz crystal as transparent electrodes that enabled to observe the cells in transmission mode with the micro CCD camera when the cells were seeded on a surface of the quartz crystal.