Abstract

With scientific advances in biological research, cultured cells are being used as simulations of living organisms with the goal of building synthetic biosystems for research in the fields of bioengineering and medical applications. One of the most important of these uses is studying the adhesion and separation of cultured cells on surfaces. In addition, studying their life and death is important as a result of exposure to stimuli and chemical pressures. The study of cell morphology is important in these studies, as it shows changes in the shapes of cells as a result of the influence of stimuli or inhibitors on them. Moreover, cultured cells differ in their shapes depending on the type of surface and type of extracellular matrix (ECM).

In order to observe cell morphology and the effects of ECM in response to an antitumor reagent for cultured cells and monitor the attachment process, I have developed a quartz crystal microbalance (QCM) system with a micro-CMOS camera and a Peltier device. The system enables microphotograph imaging and temperature controlling simultaneously with QCM measurement, where I have improved imaging and measurement performance to get more repeatable data. The lighting unit and electronic focus control of the lens have been added, and linked to the thermoregulatory unit for precise control and rapid temperature change. This system was used in a CO_2 incubator. The electrodes of QCM were made after optimizing and tuning their working conditions from transparent indium tin oxide (ITO) electrodes with a thin thickness and high transmittance, which enable to obtain a transparent mode microphotograph.

I have studied different types of ECM that will contribute to cell attachment on ITO electrical surfaces to monitor and analyze changes in cells. After forming poly-L-lysine (PLL), collagen, and thermoresponsive polymer poly(N-isopropylacrylamide) (PNIPAM) layers on QCM surfaces, human hepatoma cell line (HepG2) cells were cultured at 37 ˚C. The attachment process of cells cultured on poly-L-lysine (PLL), collagen, and thermo-responsive polymeric polymer (N-isopropylacrylamide) (PNIPAM) were studied. At the same time, micro photographs of the cells were recorded to observe the morphological changes.

During cell attachment process, resonance frequency decreased and resonance resistance increased, which meant that cells attached on the quartz crystal surface. In case of PLL and collagen the resonance frequency and resonance resistance showed the attachment finished within 24 h. This corresponded to the cell image observed with the micro-CMOS camera. To determine the relationship between change of resonant frequency and cell number on a QCM, a cell-cell interaction model has been proposed using the binomial equation in the model mass effect. In the proposed equation, the variable mass ratio (variable mass: m_v / steady

mass: m_s) was 1.75, and the close contact probability of cells was 1/2400, which was determined to correct the resonance frequency modeling curves of the experimental results. Analysis of the fitting curves showed that the curves fitted to the first order lag response and the time constants of the first order lag response were 11 h for PLL, 16 h for collagen and 38 h for PNIPAM films. These findings were supported by photographic images showing wider spread cells on PLL and collagen than PNIPAM. The response of cells on PNIPAM was measured during a thermal cycle from 37 to 20 °C and then from 20 °C to 37 °C.

For the cell response process after injection of the antitumor cisplatin, two response steps were observed in both QCM data and microscopic images, where the cells loosened in the first step and shrank in the second step. Log-normal distributions were applied to match both steps by the resonant frequency responses of both processes. The measured curves and the modeling curves are in good agreement with QCM data. By fitting results, I obtained survival rates of 0.1 and 0.2 when the cisplatin concentrations were 83.3 and 16.7 μmol/L, respectively.

In this thesis, I have developed a measurement system that allows monitoring of cells in a CO_2 incubator. Using this system, I observed how the cell interacts with ECM and attaches to surfaces, as well as the structural changes associated with these interactions. In addition to monitoring the effects of chemical and stress stimuli, and analyzing the physical properties of cells, transition of the state of cytoskeleton and antitumor drag activity was measured. Moreover, fitting curves of the model equations were investigated by matching them with the experimental curves of the resonant frequency change. The resonance frequency analysis could provide analysis of mass, viscosity, life and death of cells that are difficult to obtain through microscopic measurements alone.

When using traditional measurement methods for determine cell death, only the state of an elapsed time is measured since the measurement is performed after a certain period of time has passed. Similarly, these methods cannot show the progress or status when more time has passed. On the other hand, the method of this study is possible to measure how the morphology is continuously changing. In addition, the method in this study is also possible to analyze the mechanism of cell change. Through this system, it can be used to analyze the cell adhesion process of various ECMs. Also, it can be used to analyze changes in cell responses over time and evaluate the rate of cell death that were not able to do before.