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In-situ monitoring of leukemia cell death by infrared spectroscopy

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1. Introduction

During recent years cell-based assays have attracted much attention for drug discovery, bioprospecting and environmental assessment of chemicals. Since cellular responses are highly sensitive to trace amount of toxins, cell viability and toxicity assays are particularly important for screening biological active substances.[1, 2] Conventional multi-step biochemical assays evaluate cellular responses at every scheduled time point after destruction of the cells. On the other hand, several techniques have been reported for real-time monitoring of cytotoxic effects, such as impedance method,[3, 4] quartz crystal microbalance,[5] photonic crystal sensor[6] and Fourier transform infrared (FTIR) absorption spectroscopy.[7] Especially, FTIR spectroscopy has the potential to provide information on multiple analytes without disturbing the biological samples. In the present study, we report on a method for *in-situ* observation of cell death using FTIR spectroscopy in the multiple internal reflection (MIR) geometry with a Si substrate as an MIR prism. MIR-FTIR provides an ideal optical configuration to combine sensitive detection and aqueous-solution phase measurements. As a model sample, we used human promyelocytic leukemia (HL-60) which are floating cells and do not adhere to a surface. To maintain cell activities, the temperature and humidity in acrylic chambers was maintained at 37 °C and >80%, respectively. After confirming the growth of HL-60 cells in the acrylic chamber, the cells were exposed to cytotoxic agent and the process to cell death was monitored with MIR-FTIR spectroscopy. We demonstrated that HL-60 cells grow in our FTIR system and the absorption intensity of amide II band is useful for measuring the cell cytotoxicity.

2. Material and methods

A Si prism with an optical path length of 15 mm was fabricated and used as an MIR prism. FZ Si (100) (5250-7050 Ω cm, double side polished, 450 μm in thickness) wafer was anisotropically etched to make the structure as depicted in Fig. 1(a). The prism was set on the bottom of an acrylic chamber. Infrared light beam from an interferometer (BOMEM MB-100) was focused at normal incidence onto one of the two anisotropic etch pits of the Si prism, and penetrated through the Si prism. The light that exited the Si prism through the other bevel was focused onto a liquid-nitrogen cooled mercury-cadmium-telluride (MCT) detector. HL-60 cells (Riken cell bank) were cultured in RPMI 1640 medium (Gibco), supplemented with 10% heatinactivated Fetal Bovine serum (Sigma), penicillin (50 I.U./ml) and streptomycin (50 g/ml) (denoted as culture medium) at 37˚C in a humidified 5% CO_2 atmosphere in an incubator.

A schematic view of the present FTIR system is shown in Fig.1(a). To maintain cell activities, the cell culture condition inside the acrylic chamber was controlled to be 37 ^{o}C in a humidified 5% CO_2 atmosphere, as in a CO_2 incubator. Since the FTIR sample room needs to be purged with air which was freed from water and carbon dioxide vapor, a humidified gas mixture containing CO_2 was introduced to the chamber through silicone tubes. A gas mixture containing 5% CO_2, 21% O_2 and 74% N_2, was humidified by passing through a hot water bottle via a bubbler. The humidified gas passes through a water trap prior to entry into the FTIR sample room to minimize condensation dropping down into the chamber. The temperature of the water trap was maintained at ~37 °C using a thermo controller. Another thermo controller was attached to the chamber. The temperature in the chamber kept constant within the error of ±0.5 °C over the whole time range examined. The humidity of the gas mixture in the outlet of the chamber was also monitored with the humidity sensor. Prior to introduc-

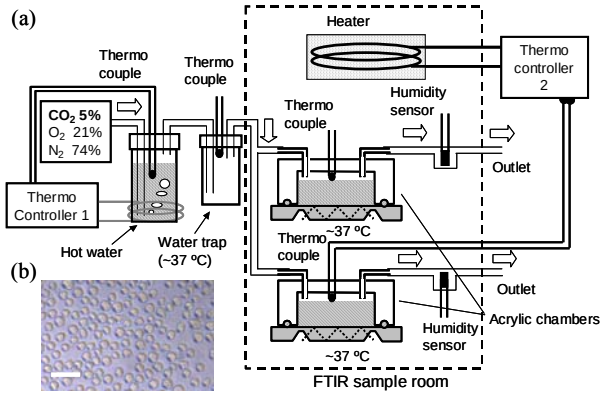

Fig. 1 (a) A schematic view of the FTIR sample room which controls temperature and humidity. (b) The inset figure shows a photomicrograph of HL-60 cells incubated for 24 h in the acrylic chamber placed in the FTIR sample room. Scale bar: 50 μm.

tion of the HL-60 cells into the chamber, the temperature in the chamber was controlled to be 37±0.5 °C and the humidity in the outlet of the chamber to be more than 80%.

Since cell activities and cell cycles varies among different sample batches, for cytotoxic assays, it is preferable to compare IR spectra between drug-treated and control cells measured at the same time under identical conditions. For this purpose, two acrylic chambers were mounted on a movable stage, whose position was controlled automatically by using four-axis stage controller and a computer.

3. Experimental results

Fig. 1(b) shows a micrograph of HL-60 cells after 24-h incubation in the acrylic chamber. Nearly all of the cells showed normal morphology with a circular smooth edge and bright shine. Growth rate of the cells in the chamber was almost same as that of the cells incubated in a CO_2 incubator. These results suggest that the cells kept in the chamber are viable in terms of morphology and proliferation even after 24 hours.

Next, the cytotoxic effect of Tween20 on HL-60 was monitored with MIR-FTIR spectroscopy. Tween20 is a surfactant which is often used in cytotoxic test assay for killing all the sample cells.[8] The suspension of HL-60 cell (~1.3×10^5 cells/ml) was transferred to the two acrylic chambers. Then, Tween20 was added to one of the chambers, while the culture medium was added to the other chamber for a control experiment. As shown in Fig. 2, the MIR-FTIR spectrum of the cells treated with Tween20 (line i) showed intense peaks centered at 1655 cm^{-1} and 1548 cm^{-1}. On the other hand, control cells did not exhibit such peaks (line ii). Tween20 itself exhibited no absorption peaks in this region (data not shown). The peaks at 1655 cm^{-1} and 1548 cm^{-1} were due to the C=O stretching (amide I band) and N-H in-plane bending and C-N stretching (amide II band), respectively.[7, 9] It is well known that Tween20 induces destruction of cell membranes to induce cell lysis. Therefore, it is suggested that the observed increase in the peak intensity of the amide I and amide II bands is due to the leakage of cytoplasmic proteins. Since the strong liquid water absorption overlap the amide I band, variations of liquid water absorption sometimes appeared in the region of amide I band as shown in the line (ii) of Fig. 2. Therefore, the amide II band reflects more accurately the total protein contents from the cells.[7]

In addition, the time course of the peak intensity of the amide II band was investigated. When the cells were treated with Tween20, the peak intensity of the amide II band rapidly increased and reached plateau within 1 h. (data not shown) No viable cells were counted after 1h exposure to Tween20, which is consistent with the results obtained with MIR-FTIR spectroscopic measurements. These results indicate that cell cytotoxicity can be monitored by the absorption intensity of amide II and our method has the potential for application to a real-time cell cytotoxic assay.

4. Conclusion

We have developed a real-time method for monitoring cell death by using MIR-FTIR spectroscopy. To maintain cell activities, the temperature and humidity in the acrylic chambers in the FTIR sample room was maintained at 37 °C and >80%, respectively. We have confirmed the cell viability and proliferation of HL-60 cells after 24-h incubation in the chambers. The cytotoxic effect by Tween20 was in-situ monitored with MIR-FTIR spectroscopy. It was demonstrated that the cell death can be monitored in-situ by analyzing the amide II peak intensity. Thus our method can be applied for in-situ observation of various cellular activities, such as cell proliferation, apoptosis, necrosis and differentiation.

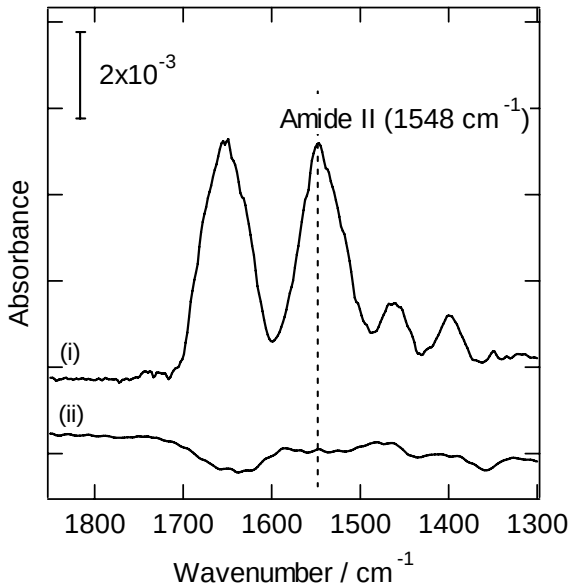

Fig. 2 MIR-FTIR spectra of HL-60 cells obtained 5 h after (i) incubation with 0.2 % Tween20 and (ii) incubation with cultured medium as a control experiment.

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