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生命科学

ラマン顕微鏡を用いた肝実質細胞内の薬効分布の無標識イメージング

■ Introduction

Human liver plays a central role in biotransforming and clearing foreign chemicals and is vulnerable to the toxicity from these chemicals. Adverse effect on liver function has been a leading cause of drug withdrawal and non-approval. In most cases, drugs that cause severe adverse effect on liver function have not been discovered in the preclinical screening step including cell and animal test. Establishment of a rapid and efficient method to investigate the drug effect on liver function in the preclinical discovery phase is a key factor to accelerate the drug development process.

Conventionally, end-point analysis of drug effect on hepatocytes, such as cell toxicity assay and enzyme activity assay, was performed. However, end-point analysis is an invasive method, which is time and labor consuming. Moreover, direct response of cells to the drug treatment cannot be monitored using this method. A non-invasive method, which can facilitate the real-time monitoring, is necessary to provide more detailed information of drug response. Recently, Raman microscope has attracted more attentions in the biological study as a label-free technology to visualize biological molecules without a fluorescent label. Applying Raman microscopy in drug discovery has a great potential to accelerate the drug development process.

■ Materials & Methods

1. Cell culture

The differentiated hepatocytes, HepaRG cells (Biopredic International), were thawed and cultured on quartz substrate in 35 mm dish according to the supplier's protocol. HepaRG culture medium was renewed on day 3. HepaRG cells were treated with the drug on day 5 and day 6. Cells on Day 7 were observed by Raman microscopy.

2. Raman observation

Raman spectra of HepaRG cells were obtained using a slit-scanning Raman microscope with 532 nm excitation laser. The laser beam is shaped into a line by a cylindrical lens and focused on the sample at the microscope stage by a 60× objective lens. The Raman signals from the sample are collected by the same objective lens, then pass into a spectrograph through a 532 nm long-pass edge filter and are then detected by a cooled CCD camera.

■ Results & Discussion

Raman spectra obtained from the HepaRG cells with or without drug A treatment were plotted in Fig. 1. Each spectrum was averaged from hepatocyte region. Main Raman peaks were indicated by black arrows. Most main Raman peaks including four peaks assigned for cytochrome c (750, 1130, 1315, 1585 cm^{-1}) were observed same between the two spectra. A Raman shift appeared at 1639 cm^{-1} after treatment of the drug A. Drug effect was visualized by reconstructing heat-map images from the distribution of the Raman scattering intensity at 1639 cm^{-1}. Cells with drug treatment showed higher level of drug response and different distribution compared with control cells. This result demonstrated the potential of Raman microscopy in visualizing the drug response of living cells.

■ Acknowledgements

This work was supported by AIST-Osaka University Advanced Photonics and Biosensing Open Innovation Laboratory (PhotoBIO-OIL).

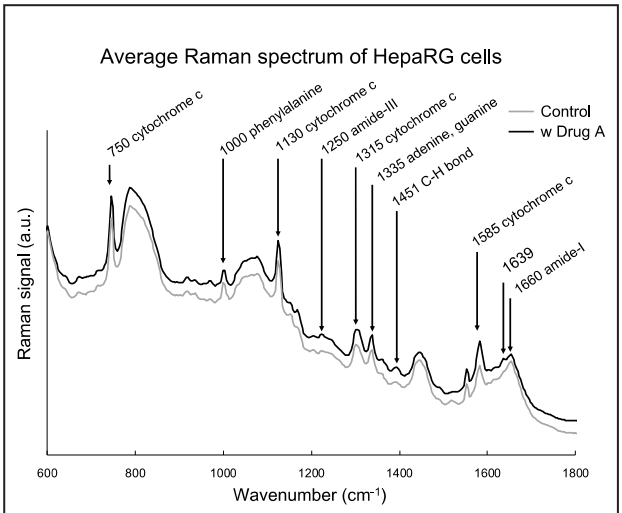

Fig. 1 Average Raman spectra of HepaRG cells.
Grey line: control cells; Black line: cells treated with drug.

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■キーワード： (1)ラマン顕微鏡
(2)薬効分布
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