Apoptosis study of the Macrophage via Near-Field Scanning Optical Microscope

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Abstract. The cell apoptosis phenomenon was studied by traditional optical microscope with much lower resolution and also observed by Atomic Force Microscope (AFM) with nano-resolution recently. They both detect the cell apoptosis through the change of cell topography.

In this study, the cell apoptosis was investigated via Near-Field Scanning Optical Microscope (NSOM). The cell topography, with nano-scaled resolution, and its optical characteristics were observed by NSOM at the same measurement scanning. The macrophage was chosen as the cell investigated. To understand the cell apoptosis process is the goal set for the research. The apoptosis process was related to the variations of the optical characteristics of the cell.

Keywords: cell apoptosis, Near-Field Scanning Optical Microscope, Atomic force microscopy, macrophage, optical characteristics

Introduction

In this study, we use NSOM, Near-field scanning optical microscope, to observe cell apoptosis. There are three advantages of NSOM, observing in normal environment, observing in nanometer scale resolution, and observing in non-contact mode. Apoptosis is one of the main types of programmed cell death (PCD), and involves an orchestrated series of biochemical events leading to a characteristic cell morphology and death. For observing apoptosis, the cell should be observed in the culture solution.

There were several researches to observe the cell morphology. Kerr, et al., observed the change of cell structure in 1972 [1]. Guangyong, et al., observed the influence of surrounding cells in the observed region to the cell morphology using AFM [2]. Four kinds of microscopes were tried to observe the cell morphology. They are optical microscope with lower resolution than required, TEM with harmful high temperature caused in the sample chamber, STM with difficulty to work under liquid, and AFM with difficulty to observe real morphology of living cell. So NSOM is another attempt to do the job.

Experiment

The cell volume is changing under apoptosis. Observing the cell morphology is a way to understand the apoptosis. Two measuring modes of NSOM are attempted to observe the cell morphology, the shear force mode and the optical transmission mode. The NSOM shear force mode is very close to the AFM force measurement but with normal force used instead. The schematic structure of NSOM is shown in Figure 1. For shear force mode, the NSOM probe is under resonant oscillation and approaching to the cell. Due to the Van Der Wals interaction, the amplitude of oscillation varied depending on the distance between probe and sample. The oscillation signals are transformed into the morphology image accordingly. Besides, the shear force mode would much reduce the possibility of damaging the cell than the AFM normal force mode.

For internal variations due to apoptosis, cell was observed by optical transmission mode of NSOM. The transmitted laser signal could be collected at the same time when morphology image is observed. Transmitted laser signal varied according to the internal
changes of the cell. The apoptosis could thus be observed.

The observing environment was controlled to keep the living cell as long as possible. The temperature and humidity were kept in 22°C and 40% respectively. Two kinds of images were observed, cell morphology and the transmitted laser signal under cell apoptosis.

**Result and Discussion**

Cell morphology images are listed in Figure 2. Figure 2(a) is the cell morphology for cell leaving the culture solution for one hour. Fig. 2(b) is the cell morphology for cell leaving the culture solution for two hours. Fig. 2(c) is the cell morphology for cell leaving the culture solution for three hours. The cell thickness was varying as cell under apoptosis process. The thicknesses for Figure 2(a), Figure 2(b) and Figure 2(C) are 2.89μm, 2.11μm and 1.81μm respectively. The cell collapse was observed by the NSOM.

Transmitted laser signals under cell apoptosis are listed in Figure 3. Figure 3(a) is the transmitted laser signal for cell leaving the culture solution for one hour. Figure 3(b) is the transmitted laser signal for cell leaving the culture solution for two hours. Figure 3(c) is the transmitted laser signal for cell leaving the culture solution for three hours. The transmitted laser signal was varying as cell under apoptosis process. The intensity of the transmitted laser signals for Figure 3(a), Figure 3(b) and Figure 3(C) are 0.51V, 0.63V and 0.66V respectively. The intensity is rising as cell collapse.

**Conclusion**

Through the cell morphology images and the laser images of NSOM, three conclusions could be drawn. First, the change of cell morphology could be observed by NSOM and thus the cell apoptosis. Secondly, changes inside the cell due to apoptosis could be observed by observing the transmitted laser signals. Finally, observing variations on cell morphology and transmitted laser signals are possible means to disclose the apoptosis process. Up to now, NSOM is a very promising tool for researches of this kind.

**Acknowledge**

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**References**

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Fig. 1 Schematic structure of NSOM

Fig. 2 Cell morphology images
Fig. 2(a), Fig. 2(b) and Fig. 2(c) are cell morphology images for cells leaving the culture solution for one hour, two hours and three hours respectively. The thicknesses of Figure 2(a), Figure 2(b) and Figure 2(c) is 2.89$\mu$m, 2.11$\mu$m and 1.81$\mu$m respectively.

Fig3 Transmitted laser signals
The intensity of the transmitted laser signals for Figure 3(a), Figure 3(b) and Figure 3(C) are 0.51V, 0.63V and 0.66V respectively.