学　位　論　文　内　容　の　要　旨

博士の専攻分野の名称　　博士（情報科学）　　氏名　蔡 萍根

学　位　論　文　題　名

Study on spatial and temporal variations in single cell rheology measured by atomic force microscopy
（原子間力顕微鏡による単一細胞レオロジーの時空間変化に関する研究）

Living cell is a dynamical system with the capability to alter its viscoelastic or the so-called rheological property in response to external stimuli. This capability is mainly originated from the actin filamentous structure, which spatially and temporarily changes in cell cytoplasm. The advanced micro-rheology technique revealed that the averaged complex shear modulus G* of cells characterizing their rheological properties shows a power-law behavior as a function of frequency or time even the actin filamentous structures dynamically change. Besides the averaged quantity of G*, the micro-rheology measurements succeeded to directly measure spatial, temporal and cell-to-cell distributions. However, it has not been fully understood to what degree these distributions are precisely quantified, which is important in the statistical evaluation of pharmacological treatments and the comparison of different cell states.

In this study, the relationship between cell-to-cell variation and spatial variation or between cell-to-cell variation and temporal variation in cell rheology was investigated using atomic force microscopy (AFM) combined with a microarray technique. Firstly, the cell-to-cell variation was investigated by the modifications of the measurement location, the F-actin structure and the interaction between actin and myosin. Secondly, the temporal variation was studied by the modification of F-actin structure of single mouse fibroblast cells.

In chapter 2, the experimental principle including experimental material, method and model was introduced.

In chapter 3, the changes in cell-to-cell variation by the modifications of the measurement location and F-actin structure in cells were investigated. The frequency dependent component of cell–cell variation of cell rheology was estimated as the inherent cell-cell variation by subtracting the experiment error from cell-to-cell variation. The results showed that the inherent cell–cell variation can be reduced greatly by disrupting F-actin networks, by probing at locations, and by measuring at high loading frequencies.

In chapter 4, the relationships among power-law parameters for cell-to-cell variations in the storage moduli G' were clarified by investigating the cell-to-cell variations of single cells in different microarray samples. The results showed that the spatial dependence of the inherent cell-to-cell variation is preserved even the spatial heterogeneities of G' are changed depending on the cell samples. The invariance in the frequency-dependent cell-to-cell variation indicates the robustness of AFM for the mechanical diagnosis of single cells.

In chapter 5, the effect of the interaction between F-actin and myosin on the cell-to-cell variation in single cell rheology was investigated. The results indicated that the actin-myosin interaction regulates

the heterogeneities of CSK structure that enhance the cell-to-cell variation in cell rheology.

In chapter 6, the temporal variation of rheological properties of single mouse fibroblast cells under a confined condition was elucidated by tracing a long-time evolution of single cell rheology measured at the same locations. Based on a power-law rheology model, the result suggests that rheological properties of cells in the confined condition follow an ergodicity condition, in which cell mechanical state transits in all possible states with a relaxation time of several and tens minutes so that all the possible mechanical states of the ensemble one are traced.

In summary, the inherent cell-to-cell and temporal variations in cell rheology, for the first time, were characterized by subtracting experiment error. The result revealed that the inherent cell-to-cell variation exhibits a spatial dependence and was regulated by the actin-myosin complex organization. Moreover, the relationship between cell-to-cell variation and temporal variation was discussed in which the transition mechanism of the rheological state of single cell has been revealed. This study is of great practical value in the statistical evaluation of pharmacological treatments, the comparison of different cell states and the mechanical identification and sorting of cells.