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主 論 文 の 要 旨

論文題目 **Measurement of the Mechanical Property of Unicellular Organism Using Microfluidic Chip and Atomic Force Microscopy**
(マイクロ流体チップと原子間力顕微鏡を使用した単細胞生物の機械的特性の測定)

氏　　名 **CHANG　Di**

論 文 内 容 の 要 旨

This thesis focusses on the measurement of the mechanical property, such as Young's modulus, of unicellular organism. Unicellular organisms, such as cyanobacterium and yeast, play important roles in the study of biofuel, gen analysis, protein production and so on. The mechanical property of the cell is different according to the environment condition or the cell gene. Thus, it is considered as an important parameter that present the cell living condition. But the diameter of the cell is as small as several micrometers, which makes it difficult to operate the cell. And it is also difficult to measure the cellular deformation and the applied force because of their small size.

Firstly, to overcome the difficulties caused by the small size of the cell, a measurement system is constructed. This system consists of a robot integrated microfluidic chip and an optical tweezers system. The optical tweezers allow us to manipulate the cell. The microfluidic chip integrated with a force sensor and a pushing probe allows us to deform a cell. And then, we can measure the deformation and applied force accurately using the chip. Measuring the stiffness of single cyanobacterium cell (*Synechocystis* sp. strain PCC6803) whose diameter is less than 2 μm is realized using the system. The Young's modulus of single cyanobacterium cell in different osmotic environment conditions are measured. Two types of cell are measured, the wild type (WT) and the *ΔmscL*. The wild type is not genetically modified. The *ΔmscL* is genetically modified and lacks a specific kind of ion-channel called mechanosensitive channel (MscL). It is confirmed that the *ΔmscL* and WT have different Young's modulus in different osmotic conditions.

Secondly, for the purpose of comparing with conventional research which utilize the atomic force microscopy (AFM) to study cell mechanical property, the measurement of single yeast cell using atomic force microscope and microfluidic chip are studied. There are lots of studies which studied the property of yeast cell using AFM. The Young's modulus of single yeast cell are measured using AFM with a sharp tip and a customized flat tip respectively. The sharp tip indents the cell and measure the local property of the cell, which is a conventional research method. While the flat tip compresses the whole cell and evaluate the Young's modulus of the cell as a whole. The Young's modulus measured by microfluidic chip and AFM with flat tip are similar, which prove the reliability of this method. While the Young's modulus measured by sharp tip and flat tip are quite different with each other. This might because the sharp tip measures stiffness of the local position on the surface of a cell, while the flat tip evaluates the stiffness of the whole cell. Other researches which studied the Young's modulus of yeast cell using AFM with sharp tip have reported the Young's modulus in the same order. Thus, the sharp tip result in this research is considered to be reliable. The two different tips evaluating the Young's modulus of a yeast cell in different ways. And both of them are suitable for evaluating the Young's modulus of yeast cell.

In Chapter 1, background and concept of this research is introduced. A unicellular organism is an organism that consists of a single cell. It contains lots of creatures such as cyanobacteria, *Escherichia coli*, and yeast. There are lots of studies about unicellular organisms in different fields. For example, cyanobacteria are a kind of photosynthetic bacteria which live in a wide variety of moist soils and water. It has been used as a model microorganism in a wide range of studies in areas such as photosynthesis, biofuel, and environmental stress adaptation. *Escherichia coli* is a kind of facultative anaerobic, rod-shaped, coliform bacterium of the genus Escherichia. It is widely studied in the field of ecology, public health, and protein production. Yeast is a kind of eukaryotic which is widely used in in studies involving protein production, gene analysis, cell cultivation.

In Chapter 2, the measurement system used to measure the mechanical property of a single cyanobacteria cell is proposed. The concept of the measurement system, microfluidic chip design and fabrication, and the calibration of the spring constant of the beam sensor integrated on the chip are introduced. To measure the mechanical property of a single cell with small size such as cyanobacteria, a measurement system which combines optical tweezers and microfluidic chip is constructed. The optical tweezers could apply a force in the order of pN, which is used to trap the target cell and transport it to the gap between the gap between two probes. And the robot integrated microfluidic chip is used to compress the target cell and sense the reactive force. it is necessary to deform the cell to measure its stiffness.

In Chapter 3, the measurement process and experiment data of two types of cells in three different conditions are shown. While the diameters of the cell have no obvious difference. The cells used in this thesis are the model cyanobacterium *Synechocystis* sp. strain PCC6803. Two types of the *Synechocystis* cells are measured, the wild type (WT) (without genetic modification) and MscL-defective mutant (*ΔmscL*). As a result, there is a significant difference for *ΔmscL* between normal and low osmolarities. While t-test for WT between normal and low osmolarities shows that the difference is not significant. The diameter of each cell is also summarized. The diameter of each cell is also measured. The distance between the pushing and sensing probe is recorded when the sensor started moving during the compression process. The distance is regarded as the diameter of the cell when the sensor starts moving. Although the displacement measurement is accurate using this chip, no clear difference between cell types or osmotic concentration is observed.

In Chapter 4, a typical unicellular organism, yeast, is measured using AFM and the microfluidic chip. The yeast cell is measured by AFM in two different methods. In one method, the yeast cell is indented by a sharp tip to measure the local stiffness of the measurement point. In the other method, the yeast cell is compressed as a whole, and the Young's modulus of the whole cell is measured. Microfluidic chip method proposed in Chapter 2 is carried out and compared with the two methods using AFM. Yeast is a type of eukaryotic cell, and it is widely used in studies involving protein production, gene analysis, cell cultivation, and biofuels. AFM is a type of scanning microscopy technique. This is a method that is widely utilized for evluating the mechanical properties of cells or tissues. But the use of a sharp tip has some disadvantages in the case of yeast cell indentation. A method of measuring Young's modulus of a single yeast cell using a cantilever with a flat tip whose surface area would be larger than the cell is proposed in this chapter. The procedure involves compressing the target cell between the flat cantilever tip and the substrate. Thus, the Hertzian model is effective for the analysis.

PDMS beads are measured to determine the measurement error occured when using the AFM cantilever with the sharp tips. The measurements on PDMS beads and single yeast cells are performed after immobilizing the respective samples on the glass bottom dish (substrate) and compressing them using the AFM cantilever with the flat tip. Then, measurements are performed on single cells of a wild type strain of budding yeast, *Saccharomyces cerevisiae* BY4741, which is a widely used laboratory strain. This method employs an AFM cantilever with a flat tip whose area is larger than a single yeast cell. The cells are compressed between the bottom of the flat tip and the substrate, and Young's modulus of the whole cell is calculated to evaluate its mechanical property. For comparison, this property of the cell is also measured using the microfluidic chip with two probes, based on the method proposed in Chapter 2. Also a sharp tip is utilized to measure the mechanical property at the surface of a single yeast cell to compare the result between sharp tip measurement and flat tip measurement.

In Chapter 5, this thesis is summarized, and the future work is discussed. In summary, this thesis proposed a measurement system which is capable of measure small unicellular organism with a size as small as 2 μm. Experiments on yeast BY4741 are also carried out. The measurement result using AFM with sharp tip, AFM with flat tip and the robot integrated microfluidic chip shows that Young's modulus of the whole cell and the cell surface is greatly different with each other. Measuring the mechanical property of the whole cell give us another view to understand the cell structure.

In the future, the research could be developed to several directions. One direction is combining the robot integrated microfluidic chip with other microfluidic systems such as temperature control and microvalve. By combining different functions and systems, it would be able to measure the mechanical property of the target cell in different environment conditions. Another direction is measuring different types of genetically modified cells to understand more about the cell life, gene expression and environmental adaptation. Integrating the microfluidic chip with computer and coding is also a good direction. It would be able to achieve automatic measurement for cell properties. Only cells of sphere shapes are measured in this study. In the future, it is possible to measure cells with other shapes such as ellipse or thin rod. This might need to propose special force models.