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Study on the Elastic Characteristics of Living Cells using Atomic Force Microscope Indentation Technique

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Abstract: In this work, imaging and study of elastic property of the living cell was performed. The motivation of this work was to seek the possibility of exploiting Young's modulus as a disease indicator using Atomic Force Microscope (AFM) and also to gain fundamental understanding of cell mechanics for applications in medical nanorobots of the future. L-929 fibroblast adherent cell was used as the sample. Imaging condition in cell culturing media environment was done in very low speed (20 μm/s) compared to that in the ambient environment. For measuring the Young's modulus of the living cell, AFM indentation method was used. From the force-distance curve obtained from the indentation experiment the Young's modulus could be derived using the Hertz model. The Young's modulus of living L-929 fibroblast cell was 1.29 ± 0.2 kPa.

Keywords: atomic force microscope (AFM), cell, indentation, young's modulus

1. Introduction

Development of scientific technology has provided base for the development in diagnosis, medical treatment and medicine. For instance, emergence of microscope opened the new era possible to investigate minute biological samples beyond the human eye sight. Widely used medical instruments for diagnosis such as X-ray, ultrasonic diagnosis, Computer Tomography (CT) and Magnetic Resonance Imaging (MRI) were able to be applied to medical field owing to preceding development of nondestructive testing. As stated above scientific techniques largely influence the bioengineering and its medical application. As technology in nano/micro scale advances rapidly, development and research on DNA chip, Lab-on-a chip that make possible to diagnose and test with minimum amount of the sample start to expand.

Atomic Force Microscope (AFM) is widely applied in nanometer scale physics and chemistry. The strong point of AFM that can image in high resolution up to nanometer scale is a useful ability that can be applied in bioengineering research [1]. In addition, the direct force measurement is also capable with AFM. In biotechnology application, this capability was adopted to measure the binding force between receptor and ligand and unfolding force of folded protein such as titin [2].

The high resolution imaging and force measurement in nanonewton range play an important role in research on cell mechanics. For example, the effect of drugs on cytoskeletal structures of the cell was studied through investigation on the cell surface and mechanical characteristics using AFM [3]. Furthermore study on cell exploiting AFM may be applicable in medical field. The researches on elastic characteristic and viscoelastic behavior of cells have the potential to be used for new biomarkers which can detect the diseases [4]. The cells infected with the diseases change their cytoskeletal structure into an alternative structure of cytoskeletons. These changes may bring about different mechanical behavior and properties of the cells. Thus the change of mechanical properties of the diseased cell and drug treated cell can be detected.

There are a lot of efforts spent by scientists and engineers to develop nanorobots recently. One of the core applications of the nanorobots in the future is medical treatment. These nanorobots aim direct drug delivery to the cells, injecting and destroying the diseased cells moving through the blood vessels [5,6]. They function to grab, puncture and destroy the diseased cells. Thus to design nanorobots to perform these tasks properly, the mechanical properties of the cells must be studied in advance.

The study on mechanical properties can be used for medical application such as biomarker for disease indicator as well as for the fundamental research leading to the development of nanorobots. In this research the L-929 fibroblast image and Young's modulus were obtained in living state by exploiting the functions of an AFM.

2. Experimental Details

2.1. Sample preparation

The cell line used for the experiment was L-929 mouse fibroblast (ATCC®). The cells were cultured in the Dulbecco's modified Eagle's medium (DMEM) containing 10% Fetal Bovine Serum (FBS), 1% antibiotics. They were incubated in CO_2 5% environment at 37.5°C. Fig. 1 is the picture of L-929

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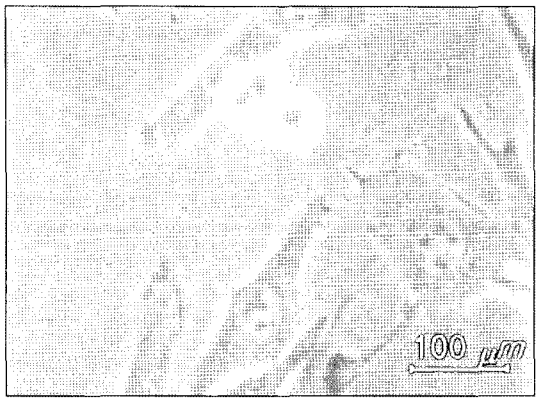

Fig. 1. Optical microscope image of L-929 fibroblast.

fibroblast taken with an optical microscope. As shown in the picture L-929 fibroblast adheres on the substrate.

For the experimental use, the cells were grown on an 18 mm diameter cover glass (Paul Marienfeld GmbH & Co. KG). The cover glass on which the cells were grown was attached to a metal block. Since the cover glass was transparent there was some difficulty in finding the sample cells using the AFM optical system. The metal block helps in investigation of the sample cells on a cover glass by reflecting the light. The experiments were conducted in the cell culturing medium to keep the cells alive. The cell culturing medium was put into the Teflon dish and the sample cells on the metal block were immersed in the Teflon dish.

For the imaging and the indentation measurement in the cell culturing medium, the cantilever holder that was made of quartz and coated with gold was applied. The cantilever used for imaging the living cell was a commercial Si_3N_4 cantilever with stiffness of 0.006 ± 0.01 N/m (Veeco Probes, OBL) and Si cantilever with stiffness of 0.12 ± 0.01 N/m (Nanosensors™. PPP-CONT) was used for the indentation measurement.

2.2. Experimental set-up and method

Fig. 2 shows the schematic of the experimental set-up for the living cell imaging and measurement. To obtain the images of the living cells the noncontact mode was used. The noncontact mode is the scanning mode that uses the change of resonant frequency due to the change of the distance between the apex of the tip and the surface as the AFM pzt scanner scans the surface. It is relatively easy to damage the sample and detach the cell from the surface as the living cells are softer than other solid samples. Thus care is needed to avoid damaging the cells. Furthermore, the scanning conditions such as scan speed should be considered carefully taking the damping effect of the cell culturing medium into account.

The indentation technique using AFM in this work gives the force-distance curve which shows the relationship between the force applied on the cantilever and the distance of cantilever movement. The normal force was applied to the cell surface using the elastic recovery force of cantilever indenting on the cell surface. The force-distance curve displays the deflection of the cantilever as the substrate moves in the vertical direction

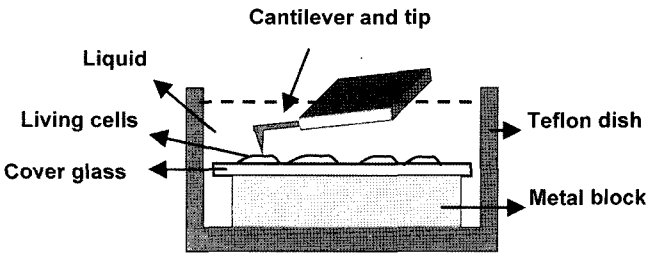

Fig. 2. Schematic of experimental set-up for living cells.

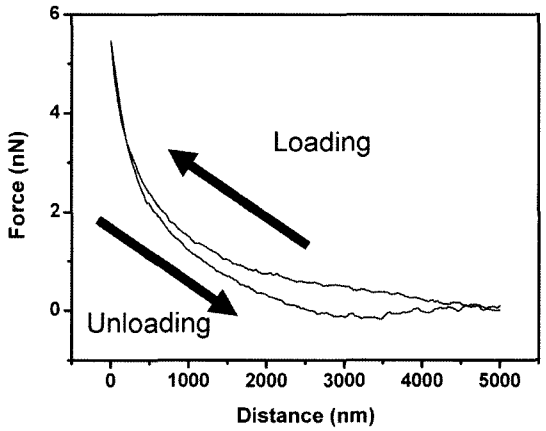

Fig. 3. Force-distance curve of L-929 fibroblast.

driven by a piezoelectric scanner. The piezoelectric scanner moves the sample substrate up and down at a constant speed adjusted by the total moving distance and time. The time is the total time for a scanner to complete the up and down motion of the sample.

Fig. 3 is the typical force-distance curve obtained for the L-929 fibroblast. The x-axis represents the displacement of the tip as it approaches and retracts from the cell surface and the y-axis represents the force between the tip and the cell. The upper line is the loading line during indentation and the line below the loading line is the unloading line during retraction. We can calculate the Young's modulus of the cell from the compression data with the acquired force-distance curve. The experiment was performed within 3 hours after the cells were taken out from the incubator to minimize the transformation of the cell due to different environment.

3. Results and Discussions

3.1. Imaging of the living cell

Using 0.006 ± 0.01 N/m stiffness and 13 kHz resonant frequency Si_3N_4 cantilever, 50 μm × 50 μm area was scanned under 0.2 Hz (20 μm/s) scanning speed with 15 kHz vibration. This scanning speed is quite low compared to that used for imaging in ambient environment. The reason for the low scanning speed is the damping effect of the cell culturing medium and to avoid damage of the cell.

Fig. 4 is the AFM data of L-929 fibroblast taken under noncontact mode. The exposure of the living cell in different environment from incubating condition for 3 hours caused

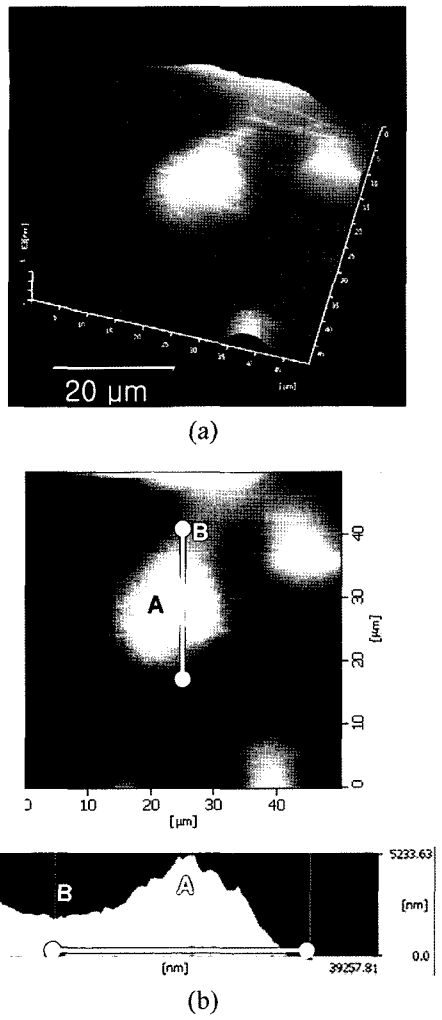

Fig. 4. (a) 3-dimensional image of L-929 fibroblast under noncontact mode and (b) 2-dimenstional image of (a) and the profile of the surface of L-929 fibroblast.

shrinking of the cell. The highest part in the center of Fig. 4(b) is the protruded part due to the nucleus of the cell. The height of the peak point is about 5.2 μm from the substrate and the height difference to the lowest part of the adhered cell is about 3.2 μm.

3.2. Young's modulus of the living cell

The indentation experiment to obtain the Young's modulus of the cell was done under 5.4 ± 0.6 nN force to minimize the damage on the cell and also to perform indentation within the elastic range. Fig. 5 is the indentation curve extracted from the force-distance curve. The vertical moving speed of the tip was 1 μm/s.

The graph shows obviously different aspect from the data typical obtained with solid samples within the elastic range. First, the force is not linearly proportional to the distance since the cell is not perfectly elastic. In addition, the loading curve and the unloading curve do not correspond to each other as shown in Fig. 3. This hysterisis originated from the viscoelastic characteristic of the cell like other biological materials.

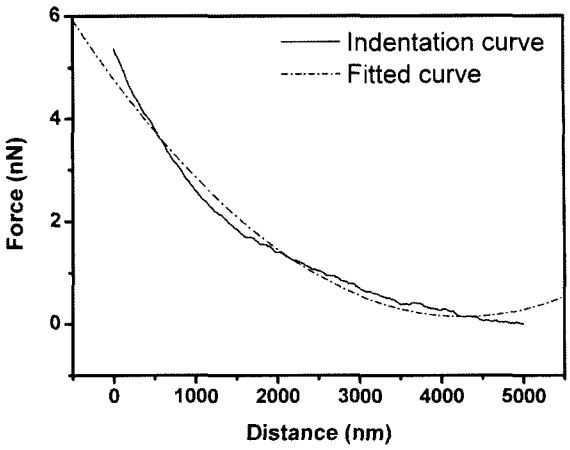

Fig. 5. Curve fitting of indentation curve of the force-distance curve.

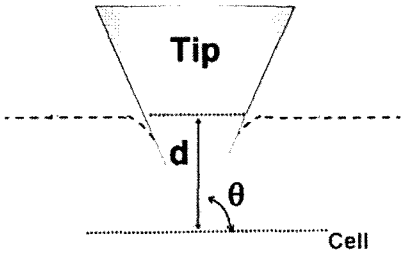

Fig. 6. Schematic drawing of the tip indenting a cell.

The Hertz model was adopted to calculate the Young's modulus. According to the Hertz model, the applied load F, indentation depth d and Young's modulus E can be related by the following equation [7].

$$F = \frac{E}{(1-\nu^2)}\frac{2}{\pi \tan\theta} d^2 \quad (1)$$

where ν is Poisson's ratio of the cell and θ is the opening angle of the cell due to tip indentation as shown in Fig. 6. The Poisson's ratio of the cell was assumed to be 0.5 and 76° for θ was obtained from the Scanning Electron Microscope (SEM) image of the tip. The cell is assumed to be an elastic half plane. Substituting all the assumed values, the equation gives the Young's modulus E if we know the relationship between F and d. Thus the force-distance curve of the cell gives the Young's modulus of the cell in the compression part of the curve. Young's modulus from the indentation data was curve fitted with least square analysis. The calculated Young's modulus of the L-929 fibroblast was found to be 1.29 ± 0.2 kPa. This value was comparable to the values of other cells obtained by other researchers [4,7,8]. Thus, the indentation method used in this work was effective in characterizing the mechanical property of the living cell.

4. Conclusion

Investigation on topography and mechanical behavior of the living cells were done by utilizing an AFM. The experiments

were done in the cell culturing medium to keep the cells alive.

The scanning conditions needed to be carefully determined for the imaging of the living cells. The noncontact mode imaging was done in very low speed (20 μm/s) compared to that in the ambient environment.

AFM indentation technique was used to acquire the force-distance curve of the living cell. The obtained force-distance curve showed viscoelastic characteristics of the living cell as was evident from the hysterisis. Also the Young's modulus was obtained from the force-distance curve of the living cell. To analyze the force-distance curve Hertz model was adopted. The calculated Young's modulus of L-929 was determined to be 1.29 ± 0.2 kPa.

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