Silicon Nanowire-Based Methodology for Quantifying Single Cell Traction Force

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Abstract – **In this paper, we present a new method, a silicon-nanowire-array based technique for quantifying the mechanical behavior of single cells, representing three distinct groups: normal mammalian cells, benign cells (L929) and malignant cells (HeLa). By culturing the cells on top of NW arrays, the maximum traction forces of different cells have been measured by quantitatively analyzing the bending of the nanowires. The elastic modulus of the as-fabricated Si-NW arrays was first measured before cell culturing. Finite Element (FEM) simulations were carried out in order to derive the relationship between the applied transverse force and the corresponding tip displacement for a Si-NW. Our study is likely important for studying the mechanical properties of single cells and their migration characteristics, possibly providing a new cellular level diagnostic technique.**

Keywords – **Silicon Nanowire Array, Cell Traction Force, Finite Element Simulation, Cellular Mechanical Properties.**

I. Introduction

The physical properties of mammalian cells is important for understanding the biological behavior of cells and for disease diagnosis. [1-3] These properties of stationary cells, especially the cell traction force (CTF), are of special interest because they are crucial to some biological processes such as extracellular matrix (ECM) generation, mechanical signal transmission, morphology and cell migration. The CTF measurements are potentially useful for understanding many important processes, such as embryogenesis, angiogenesis, histogenesis, wound healing, inflammation, cancer invasion and metastasis. [4-7]

Quantitative analysis of the mechanical behavior of a single cell traction force is an area of intensive study with copious challenges. [1-7] Several different methods have been developed for measuring CTFs in recent years. Polymerized bovine collagen was mixed with cells to form a gel disk that monitors the cell force, [3,8] but this method is unable to measure the CTF of individual cells. Thin silicone film provides another method for CTF measurements, [9] but the data analysis is rather complex owing to the involvement of nonlinear mechanics for quantifying the wrinkles of the thin film. A micropost force sensor array has been developed and widely used for measuring CTF. [8] From thousands of microposts, local traction force generated by cells can be measured along all directions for a single cell. [5,10] The diameter of the microposts is >0.75 μm and the length is adjustable. [11-12] The long micropost produces very flexible, extendable and soft beams that are easily bent, so that the measured force may not be the maximum force applied by the cell, while a short micropost can suffer from elastic elongation (or the pulling up effect) of the substrate and itself. As a result, the accuracy of the measured force may be limited by two factors, one being the density of the microposts and the other the resolution of the optical measurement technique adopted. [13-14]

Silicon nanowires (Si-NWs) have been demonstrated as unique probes for quantifying biological processes at a high spatial resolution. An integration of arrays of field effect transistors made using Si-NWs and the neuron system by Lieber presents a breakthrough approach for studying electrical signal transmissions in neuron and biological species. [16-21] In this paper, by culturing cancer cells and normal cells on Si-NW arrays, respectively, the deflections of the NWs were used to derive the lateral cell CTFs in reference to the calibrated force-displacement curve received by atomic force microscopy and finite element simulation. Our work present a new way towards single cell CTF measurement. Our results indicate that the cancer cells (HeLa, L929) have significantly larger CTFs than a normal cell, and both of them in the micro-Newton range. This study presents a nanowire-based methodology for quantifying the mechanical behavior of single cells, which could be useful for understanding the migration behavior of cells.

II. Materials and Methods

To investigate the maximum CTF that can be generated from a single cell, uniform Si NW arrays with controlled diameters have been fabricated by an aqueous electroless chemical etching of silicon wafers using a published procedure. [22] The diameter, length, and uniformity of the Si NWs were well controlled by etching conditions.

Three cell lines were used as model systems in this study, mechanocytes, L929 cells, and HeLa cells. Mechanocytes were harvested from neonatal rat liver (SD Rats). L929 and

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HeLa cells were purchased from American type Culture Collection (ATCC). Mechanocytes were cultured in RPMI-1640 medium (ATCC) supplemented with 10% fetal bovine serum (ATCC) and 1% penicillin/streptomycin (ATCC). L929 and HeLa cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM, ATCC) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Three different cell lines were incubated at 37 °C in 5%CO_2 and at a constant humidity.

Suspended cells were prepared from anchorage cells cultured in vials for 3 days. The suspensions were centrifuged and the supernatant medium with trypsinase was removed. Then cell pellets were resuspended and diluted in cell culture medium to a density of 10^6 per ml.

After checking by SEM, Si-NWs with smaller diameters either in the group of 140±16.7 nm or in the group of 280±40.4 nm (aspect ratio is 21.7±2.6) were used for the experiments, because they were more easily bent by the cells. The cleaned and sterilized Si-NW arrays were put in sterile Petri dishes immerged into cell culture medium (6 mL/dish) in advance. Suspended cells were added into cell culture medium and shaken slightly to help dispersion, and then the cells were incubated at 37 °C in 5% CO_2. The aligned Si-NW arrays were taken out of cell culture medium at 2, 6, 12, 24, and 36 h and the cells attached on them were fixed and dried, as described in previous literatures, [10,23-26]after immerging samples in 1% chlorauric acid (in DI water) for 2 h for contrast enhancement. The entire procedures are operated strictly for protecting the samples to preserve their original morphology and state. All specimens were observed and recorded by SEM.

The bending of the NWs at the edge of the cell can be clearly imaged by SEM. The degree of bending of the NWs is apparent. It is known from the mechanical behavior of the NWs that the force required to bend a NW can be derived from the degree of its bending, provided the force-displacement relationship is quantitatively established.

Atomic force microscopy (AFM) was used to quantify the mechanical behavior of the NWs following a procedure previously described. [27] From the maximum bending distance and the corresponding bending force, the elastic modulus of the corresponding Si NW is

$$E = KL^3/3I \quad (1)$$

provided the bending is in linear range, where K= f/x is the spring constant of the Si NW, f is the maximum bending force that is received from the lateral force image; x is the corresponding bending distance that could be retrieved from the topography image; L is the length of the NW; and I is the momentum of inertia of the Si NW. If the NW has a uniform circular cross-section along its length, the momentum of inertia is

$$I = (\pi/4)\cdot a^4 \quad (2)$$

where a is also the radius of the NW. The advantage of this technique is that the elastic modulus of individual Si NWs can be measured systemically and simultaneously without destructing the sample.

The elastic modulus of the as-fabricated Si-NW arrays was first measured before cell culturing. The measurement was carried by AFM (MFP-3D from AsylumResearch Co. Ltd.). After 10 group measurements using the Si-NWs that have a uniform cylindrical geometry, the average elastic modulus of Si NW was received to be 151±38.3 GPa.

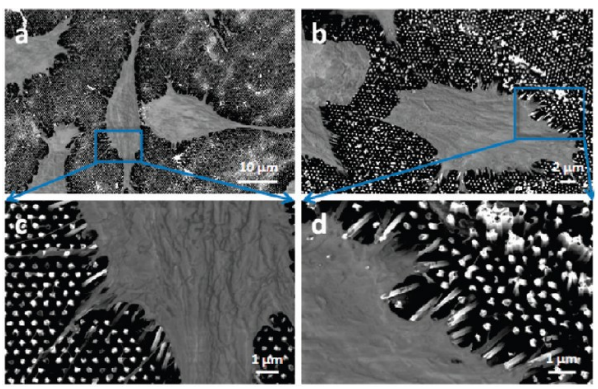

Fig. 1 Measure the lateral deflections of Si-NWs as a result of cancer cell traction force. SEM images of L929 cell cultured on aligned Si-NWs for 24 h (a, c) and 36 h. (b, d).

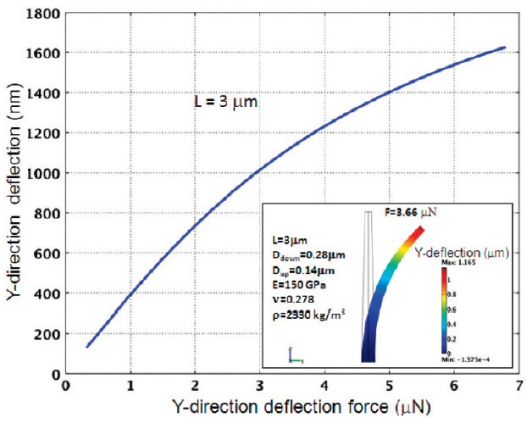

Fig 2. FEM calculated relationship between the applied force parallel to the substrate (y-direction) and the tip deflection distance of a Si-NW. The schematic diagram in the right-bottom corner shows the deformation of Si-NW. The color code represents the lateral displacement.

The lateral displacement d of a NW parallel to the substrate was measured directly from the top-view SEM image using software Image J, [28]and it will be used to derive the CTF parallel to the substrate.

To derive the CTF, we must consider the nonlinear mechanical effect of the NW because the degree of its bending is rather large. For this purpose, Finite Element (FEM) simulations were carried out in order to derive the relationship between the applied transverse force and the corresponding tip displacement for a Si-NW using the experimentally measured dimensions and mechanical parameters. Our calculation was based on COMSOL package including nonlinear mechanical effects. Considering the realistic shape of the NW, its shape was modeled as a truncated cone with a bottom diameter of 0.28 μm and a top diameter of 0.14 μm. Silicon nanowire as 3 μm length have been taken into account, in accordance with SEM characterizations. The mechanical properties for silicon were chosen as follows: Young's modulus E=150 GPa and Poisson's ratio ν=0.278. The base of the NW was fixed to a flat substrate and the force was applied in parallel to the substrate at the top surface of the NW. Fig 2 shows a calculated curve for a free-standing beam, in which the bending configuration of the beam is inset. This curve serves as the calibration for quantifying the transverse force from the measured lateral displacement of the NW tip. The results for different cells will be presented in Fig 3.

III. Results and Discussion

In this study, the Si-NWs are rigid and their Young's modules (E=150 GPa) are the highest of all the materials used for investigating the CTF, which is close to the Young's modules of bulk silicon material. The maximal CTFs of three different cells are in the micro Newton range. The average values of the CTF for the mechanocyte, HeLa cell, and L929 cell are 2.32±0.16, 2.84±0.49, and 3.48± 0.46 μN, respectively, being cultured for 24 h. Their maxima are up to 2.6, 4.0 μN and 4.76 μN, respectively (Fig 3). These three cell lines were chosen for representing the following three distinct groups: normal mammalian cells, benign cells, and malignant cells. The average CTFs of the HeLa cell and the L929 cell are increased by 22 and 50% more than that of the mechanocyte, respectively. Their corresponding maximal CTFs are increased by 53 and 83%, respectively. From this result, we may conclude that the CTFs of the L929 cells and HeLa cells are significantly higher than that of the mechanocyte, and the variation trends of the CTFs (mean and maximum) from mechanocyte to HeLa cell and L929 cell are similar. Such results are received for the first time for normal cells, benign cells and malignant cells.

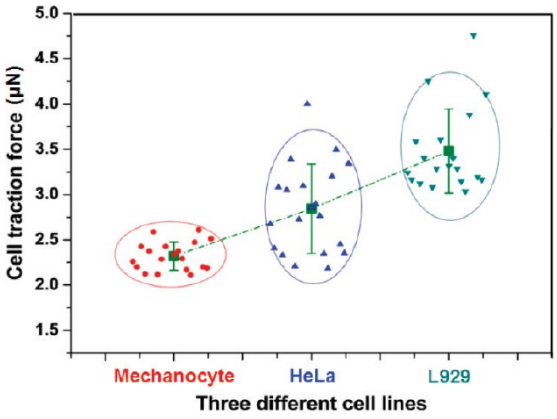

Fig. 3 The measured maximum and average CTFs of mechanocyte, HeLa cell and L929 cell, each of which was cultured for 24 h. The data points were plotted with a slight shift in the horizontal axis to show the number of data points. The green dots represent the averages of the corresponding groups. The vertical bar represents the standard deviation.

In comparison to the data reported in ref 10 using the microposts, the CTF measured by our technique is much higher. We believe that our data are more reasonable for the following reasons. First, our measurement used the nanowires with a high Young's modulus and a high aspect ratio, which were grown on a solid and rigid substrate. Such nanowires have little self-elongation in comparison to the microposts made using polymer, significantly increasing the reliability of the measured data. In contrast, microposts made using polymer on a soft substrate may suffer from elongation and a vertical pulling effect once in interaction with cells. Second, the density of the Si-NWs ($\sim 4\times10^8$ NWs per cm^2) is much higher than that of the microposts ($\sim 3\times10^7$ microposts per cm^2).[11] As a result, the cell can sink to the bottom of the microposts onto the substrate due to the large intermicro-post distance, so that the force acting on the microposts is close to their roots. The largely decreased torque acting on the microposts resulted in small lateral deflection, thus, the force was probably underestimated and the experimental error could be large as well. In our measurement, we made sure that the density of the NWs was high enough so that the cells were at the top of the NWs, which was beneficial for data analysis and the high sensitivity of the force measurement. Furthermore, the large aspect ratio and small size of our NW largely increased the degree of bending and the accuracy of measurements.

IV. Conclusions

In summary, utilizing the aligned Si-NW arrays as nanoprobes, we present a method for quantifying the maximal cell traction forces (CTFs) of three different cell lines, normal mammalian cells, benign cells (L929), and malignant cells (HeLa). By culturing the cells on a Si-NW array, the CTF of a cell results in an in-ward bending of NWs. By quantifying the force required to induce the bending of a NW based on the calibrated mechanical parameters provided by AFM and finite element calculation, the CTF of the cell acting on a NW is measured. We found that the cancer cells exhibit significantly larger CTFs in comparison to the normal cell. Discussions are presented regarding the reliability of the force data and the corresponding sensitivity. Our method and results are potentially useful for oncology, disease diagnosis, drug developing, and tissue engineering.

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