# OVERALL AND DYNAMIC ELASTICITY STUDY OF ENDOTHELIAL CELL BY ATOMIC FORCE MICROSCOPY

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## ABSTRACT

Biomechanical property of cells has been recognized to be related to many biological functions. The mechanical performance of endothelial cell is highly heterogeneous in both space and time. In this study, atomic force microscopy (AFM) was used as a nanosensor to measure the elasticity of a single cell. Given the heterogeneous and complex nature of cell mechanics, the overall elasticity of human aortic endothelial cell (HAEC) is measured in this study. In addition, the dynamic elasticity property of HAEC is also examined. Cells show increase in elasticity as scanning time increases.

## INTRODUCTION

Cellular mechanical behavior plays a major role in many general cell physiologic events such as cell differentiation [1], cell migration [2] and cell deformability [3] etc. Understanding cellular mechanics is essential for cellular biomechanics and bioengineering.

Endothelial cell, which is the innermost layer of blood vessel, is closely related to various cardiovascular diseases. Over the past few years, considerable amount of researches have been done relating to the connection between biomechanics properties of cells and human diseases, such as cancer. Mechanical property has shown to be an indication of diseases. Several studies have shown that cancer cells are more deformable than benign cells [4]. Oxidized low-density lipoproteins [5] or high plasma sodium concentration [6] will increase the stiffness of endothelial cells.

Several biophysical techniques such as atomic force microscopy (AFM), optical tweezers, magnetic twisting cytometry and micropipette aspiration have been developed to investigate the biomechanics properties of different cells. Among these techniques, AFM is one of the most suitable tools which could provide measurement for intact cells under their physical condition in vitro. High temporal and force resolution could be achieved. Furthermore, some crucial parameters such as buffer condition or temperature could be controlled in an easy manner.

The objective of this study is to develop a method to better characterize the elasticity of cells. Through force volume (FV) measurement, both topography and indentation curves were achieved simultaneously. The elasticity of cell is represented by the average elasticity of the cell main area characterized from the height of the cell. The dynamic mechanical property of endothelial cell as increasing of the scan time was also discussed.

## METHODS

### Cell culture

Human aortic endothelial cells (HAEC, Lonza) were grown in Endothelial Growth Medium-2 (supplemented with EGM-2 bulletKit, Lonza). Cells were kept in a humidified, 5% $CO_2$ and 37 $^{o}$C incubator. They were passaged and the media was changed every three days after cells reach around 70% confluent in a 100 mm cell culture dish. Cells were passaged using Trypsin-EDTA (0.05% Trypsin; 1 mM EDTA·4Na, Invitrogen). The behaviour of cells remained the same during these passages and passages 5–7 were centrifuged and collected in the 1 ml sterile freezing vial (90% Foetal Calf Serum; 10% Dimethyl sulphoxide). The freezed cells were put into liquid nitrogen container for later experiment.

### Preparation of samples

Four days before AFM experiment, one vial of cells was thawed and cultured. Matrigel was prepared ahead of time and frozen in aliquots at -20 $^{o}$C. One day before AFM experiment, take one aliquot (100 µl) and thaw on ice for two hours (or thaw at 4 $^{o}$C overnight) until matrigel liquefies. Dilute the matrigel into 700 µl cold DI water using prechilled pipet tips to prevent the gelation of matrigel. Put 4 sterilized circle cover glasses (12 mm) in the 24-well culture plate and place it on the ice. Pipet 200 µl of 1:8 Matrigel solution into each coverglass-containing well and tile the plate until the whole coverglass is covered with liquid. Pipet 100 µl of solution from each well and discard. Remove the plate from ice and plate it in the hood with half

opening. One hour later, aspirate the remaining liquid from each well. Until it dries further, rinse the cell once with HBSS and remove cells using above mentioned trypsin-EDTA solution. After centrifugation, the collected cells were dispersed into a medium of 2 ml. Place the coverglasses on a parafilm (preventing suspension running away from the covergalss) and pipet 180 µl cell suspensions on each coverglass and 300 µl on around 20 mm area at a cell culture dish. Subsequently, cells were kept in a $CO_2$ incubator for 2 h for cell attachment and refresh the medium. Cells were cultured for 24 hours for AFM measurement at the next day.

AFM experiments on living cells

All experiments were performed on a Dimension V AFM equipped with Nanoscope controller V and a fluid cell (Veeco, Inc). Silicon nitride cantilevers (from Nanoscience) with nominal spring constants of 0.03 N/m and a tip radius of around 20 nm were used. The spring constant was later determined by thermal tune method [7] ($\sim 0.035$ N/m).

HAEC cultured at the last day were taken out from the incubator. After rinsing the cells gently with pre-warmed PBS (containing $Ca^{2+}$ ang $Mg^{2+}$), cells were immersed in the PBS solution and focused on a microscopic sample stage on AFM station and adjusted against an optical axis which is the position of a cantilever tip. Cells and the optical axis were observed from a monitor screen, and a site with cells of interested was chosen by the computer manipulation from Nanoscope software (7.30, Veeco). The scan rate was set to 0.2 Hz to minimize the disturbance during the scan process.

Analysis of force curves and force volume method

For AFM force measurement, as the probe approaching and indenting the cell surface, the deflection signal of the cantilever is recorded. The relation between the indentation depth and the deflection of the cantilever was processed in Nanoscope software as extending (approaching) and retracting curves. The extending curve, usually called force distance (F-D) curve, is often used for cell elastic modulus analysis.

Several different models have been developed for the analysis of the force distance curves obtained with AFM. The classical Hertz's model [8] was used when samples satisfy several assumptions by being isotropic, pure elastic and infinitely large. Another model derived by Sneddon [9] assumes a rigid cone indenting a soft flat surface. The load and indentation depth relation is given by

$$F = \frac{2}{\pi} \tan\alpha \frac{E}{1-\nu^2} \delta^2 \qquad (2)$$

where $\alpha$ is the half opening angle of the conical tip. Figure 1 shows the F-D curve fitted by both Hertz model and Sneddon model. Hertz model assumes the indentation depth is less than the tip radius, therefore Sneddon model is used in this study for F-D curve fitting.

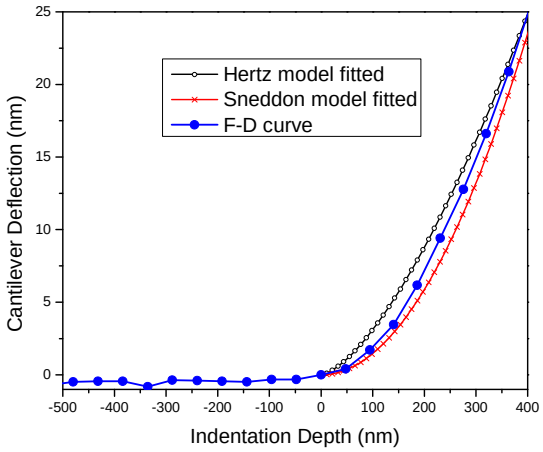
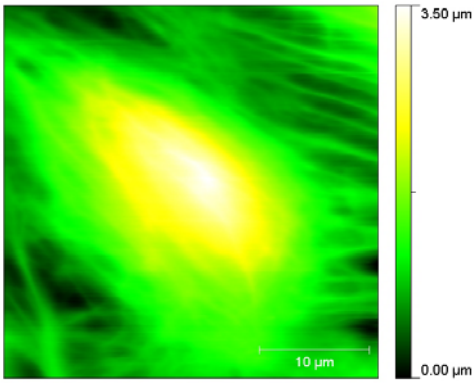



Figure 1: Force Distance curve and the fitted curves by Hertz model and Sneddon model. The blue curve is the indentation curve. The black and red curves are Hertz model and Sneddon model fitted curves respectively. Young's modulus with Hertz model fitted is 15.78±0.55 kPa and Sneddon model fitted is 16.63±0.71 kPa for this F-D curve. The spring constant of cantilever used is 0.035N/m.

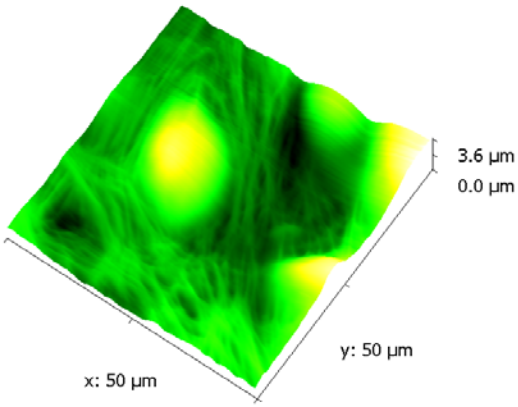
To measure the overall elasticity of cells, FV method is implemented. Firstly, the sample was scanned and an area of interest was selected for FV measurement. Then a single F-D curve is achieved from ramp plot, which is used to determine the force trigger threshold for later force volume measurement. A maximum indenting force of a cantilever was set up to 2 nN. Force plots generated at regular intervals on a sample surface are characterized as force volume imaging. If the scan area is divided into a 32×32 array (the size depends on the scan size), 1024 force-distance curves will be achieved for each force volume scan. At the same time, a surface image and adhesion force image will be obtained simultaneously. The indenting force is controlled to be very low to prevent any damage to the cell surface and to reduce any possible influence from substrate induced effects. Nonetheless, certain level of substrate effect could not be totally avoided because of the small thickness at the edge.

## RESULTS AND DISCUSSIONS

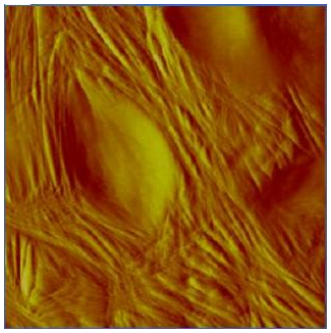
Morphology of HAEC




(a)




(b)

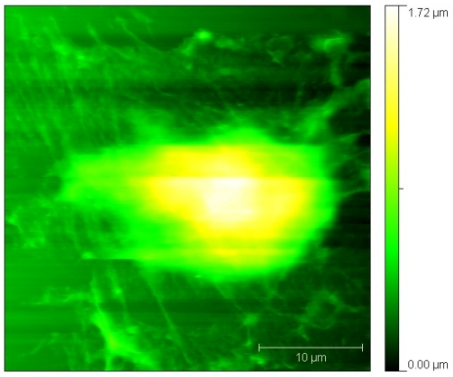


(c)

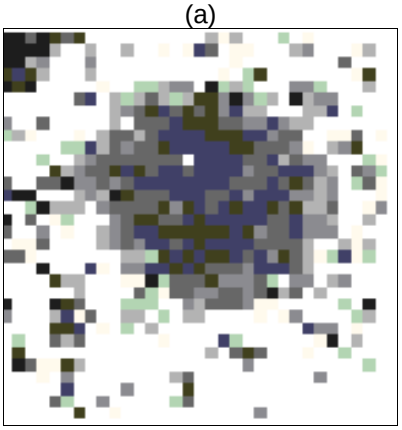
Figure 2: Contact mode morphology of human aortic endothelial cells on Matrigel substrates. (a) 2D morphology for a single cell. (b) 3D morphology for a confluent cell sample. (c) Deflection image of the same sample as (b).

On the day of AFM experiment of scanning endothelial cells, cells were first rinsed by pre-warmed PBS twice, and then cells were kept in PBS solution for AFM experiment after the system comes to a relative stable condition. Figure 2 shows the 2D image and 3D morphology of HAEC scanned by AFM apparatus. The yellow area is mainly the nuclear area which shows the larger height but not necessary to be all nuclear. In contact mode AFM, the deflection image (Figure 2(c)) on a softer sample often reveals subsurface structure more clearly than the topography Image. Figure 2(c) shows the nuclear area has a much smoother profile than the cell edge area. From the height profile and the deflection image in Figure 2, it is not appropriate to use the elasticity of any specific point from the cell to determine the overall elasticity of cells.

Force volume measurement




(a)



(b)

Figure 3 Force volume result for a single cell. (a) Topography image. (b) Elasticity map.

Figure 3 shows the force volume measurement for a single cell study. Various Young's moduli are achieved during single force-distance measurement, which means none of these could be represented as the overall stiffness of the cell.

We noticed that Young's moduli vary a lot from nuclear area to edge area. Therefore, any data from local measurement points could not represent the overall elasticity of the cell. Although the overall average of stiffness could be used as an alternative way, the effect from substrate can't be avoided at the cell edge area. Meanwhile, the cell has various heights and it is necessary to find out a method to characterize the stiffness of the cell in the whole area and make these results more meaningful. We selected criteria to define the main cell area in Figure 3, from top point of the cell to 30% of the total cell height away from the bottom. Through averaging this part, the elasticity of this cell on polystyrene surface could be achieved, 22.5±1.12 kPa.

Dynamic property of cells

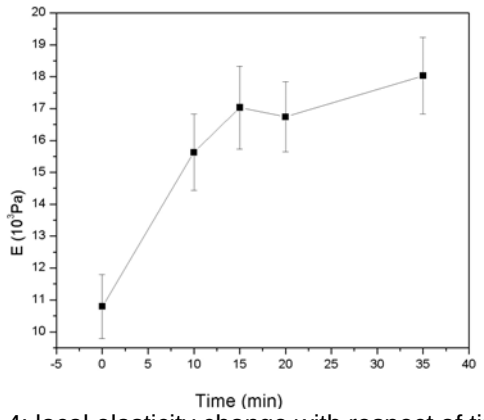


Figure 4: local elasticity change with respect of time

A small section of endothelial cell is chosen for dynamic elasticity measurement. After the cells were taken from the incubator, a section of the cell was quickly selected and the elasticity was measured. Then after several time intervals passed, the same positions are measured again. Figure 4 shows local elasticity of HAEC increases as the scan time increases during the experiment. After around 20 minutes, the elasticity comes to a relative stable level. This phenomenon is caused by the shrinkage of cytoskeleton under room temperature.

## CONCLUSION

Cell is a highly heterogeneous material and the elasticity needs to be characterized by an appropriate method. In this paper, Force volume method is used to study the overall elasticity of HAEC and the elasticity of the cell is represented through averaging the cell main area correlating to the height information. Through processing the height and elasticity information together, we could eliminate those areas which are not available for cell stiffness representation. The elasticity change of HAEC is also observed in this study. These results are beneficial for predicting the overall biomechanical property of a single cell.

## ACKNOWLEDGEMENTS

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