Journal of Biomechanics 45 (2012) 304–309

Contents lists available at SciVerse ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Characterization of cell elasticity correlated with cell morphology by atomic force microscope

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ARTICLE INFO

Article history:
Accepted 13 October 2011

Keywords:
Cell elasticity
Atomic force microscopy (AFM)
Cell morphology

ABSTRACT

Biomechanical properties of cells have been identified as an important factor in a broad range of biological processes. Based on measurements of mechanical properties by atomic force microscopy (AFM) particularly cell elasticity has been linked with human diseases, such as cancer. AFM has been widely used as a nanomechanical tool to probe the elasticity of living cells, however, standard methods for characterizing cell elasticity are still lacking. The local elasticity of a cell is conventionally used to represent the mechanical property of the cell. However, since cells have highly heterogeneous regions, elasticity mapping over the entire cell, rather than at a few points of measurement, is required. Using human aortic endothelial cells (HAECs) as a model, we have developed in this study a new method to evaluate cell elasticity more quantitatively. Based on the height information of the cell, a new characterization method was proposed to evaluate the elasticity of a cell. Using this method, elasticities of cells on different substrates were compared. Results showed that the elasticity of HAECs on softer substrate also has higher value compared to those on harder substrate given a certain height where the statistical distribution analysis confirmed that higher actin filaments density was located. Thus, the elasticity of small portions of a cell could not represent the entire cell property and may lead to invalid characterization. In order to gain a more comprehensive and detailed understanding of biomechanical properties for future clinical use, elasticity and cell morphology should therefore be correlated with discussion.

1. Introduction

It is well known that abnormal tissue stiffness is an indication of a wide range of diseases such as osteoporosis (Langton et al., 1996), atherosclerosis (O'Rourke, 1995), etc. According to the relationship between tissue stiffness and certain diseases, palpation is clinically used to detect abnormal hardness of tissue caused by diseases like breast cancer. To achieve more quantitative examinations, elastographic techniques such as ultrasound (Ophir et al., 1999) and magnetic resonance (Manduca et al., 2001) imaging methods have attracted much clinical interest in the ability to diagnose diseases based on analyzing the tissue mechanical properties.

Living cells possess specific physical and structural properties that enable them to stay alive and functional in their physiological environment. Cellular mechanics plays a major role in many cell physiological events such as cell differentiation (Fishkind and Wang, 1995), cell migration (Lim et al., 2006), and cell deformability (Dartsch et al., 1994). Deviations from the normal values of biomechanical properties of cells will undermine not only the physical integrity of the cells, but also their biological functions. Recently, studies have been done attempting to find a correlation of cellular elasticity with cell functions and human diseases, since changes in the pathophysiological properties of tissues may be manifested at the cell level. An understanding of the cellular mechanics is necessary to provide potential clinical methods that will enhance the detection, diagnosis, and treatment of disease.

Mechanical properties of cells have been studied by various single-cell techniques, such as atomic force microscopy (AFM) (Binnig et al., 1986), cell poker (Zahalak et al., 1990), micropipette aspiration (Hochmuth, 2000), scanning acoustic microscopy (Bereiter-Hahn et al., 1995), infrared laser traps (optical tweezers) (Svoboda et al., 1992), and magnetic twisting cytometry (Laurent et al., 2002). At present, AFM is one of the most advanced techniques used to study biomechanical property of cells. AFM is able to quantitatively measure mechanical properties of a cell with high spatial resolution, typically at nanometer scales in its

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doi:10.1016/j.jbiomech.2011.10.031

biophysical environment (McElfresh et al., 2002; Morrow et al., 2009). In addition, AFM has also been used to image cellular microenvironments (Zahalak et al., 1990), surface structure, and even sub-cellular structure (Raychaudhuri et al., 2009). Detection of changes in cell elasticity, measured by AFM, has been used to distinguish metastatic cells from benign cells, thus identifying disease states in cancer (Cross et al., 2007, Faria et al., 2008). In addition, AFM has been used to detect the pathological changes of cartilage cells in osteoarthritis (Stolz et al., 2009). AFM research has also been conducted on endothelial cells, which has shown that oxidized low-density lipoproteins (Chouinard et al., 2008) or high plasma sodium concentration (Oberleithner et al., 2007) increases the Young's moduli of endothelial cells. All above mentioned studies have demonstrated that AFM can be potentially developed as a diagnostic tool for detecting human disease states with fairly high efficiency and accuracy.

Although AFM is evolving rapidly in the field of biology, standard methods for achieving a more comprehensive overview of the mechanical properties of cells are still lacking. In previous studies, the local mechanical properties of cells were often measured by AFM nanoindentation techniques (Sato et al., 2000; Svoboda et al., 1992). In order to obtain statistically meaningful data, either a number of random measurements were performed at different points on one cell, or a significant number of cells were measured. In reality, the surface of a living cell has highly heterogeneous domains containing a variety of lipid, protein, and carbohydrate components. The cytoskeleton of a cell, underlying the plasma membrane, contains many kinds of proteins forming dynamic meshworks in the cytoplasm. In addition, cell morphology is highly dependent on the substrate and the cellular microenvironment. Due to the complexity of cells, variations of cell elasticity occur not only between different cells, but also at different locations on the same cell. In order to use cell elasticity as a reliable diagnostic measure to identify cellular disease in future clinical use, the objective of our study was to develop a new evaluation method that is able to determine the elasticity not only of an entire cell but also of large cell sheets such as endothelia and epithelia with consideration of cell morphology.

The objective of this study is to develop such a method to provide a more reliable quantification of the cell mechanical property that correlates cell elasticity with morphology of the cell. The effect of cell culturing substrates on the cell elasticity of HAEC is also discussed.

2. Methods

2.1. Cell culture and sample preparation

Human aortic endothelial cells (HAEC, Lonza) were grown in endothelial growth medium-2 (supplemented with EGM-2 bulletKit, Lonza). Cells were incubated in a humidified, 5% CO_2 environment at 37 °C. Culture media were changed every three days and cells were passaged after they reached 70% confluency using Trypsin-EDTA (0.05% Trypsin; 1 mM EDTA·4Na, Invitrogen). Passages 5–6 were collected in the 1 ml sterile freezing vial (90% fetal bovine serum and 10% dimethyl sulphoxide) and frozen into a liquid nitrogen container for later experiments.

For AFM experiments, cells with passage numbers 7–8 were placed on two different substrates at a density of ~300–600 cells/cm^2. Substrate A was polystyrene cell culture dishes (60 mm). Substrate B was cover glasses (20 mm in diameter) coated with Matrigel at a 1:8 dilution (Selvam et al., 2007). Subsequently, cells were incubated in a CO_2 incubator for cell attachment and the medium was refreshed after 2 h. These cells were cultured overnight for AFM measurements.

2.2. 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.). A silicon nitride cantilever (from Nanoscience) with a nominal spring constant of 0.03 N/m and tip radius of around 20 nm was used. The spring constant of the cantilever calibrated by the thermal tune method (Hutter and Bechhoefer, 1993) was 0.0206 N/m. HAECs were gently rinsed with pre-warmed (37 °C) phosphate buffer saline (PBS) solution (containing Ca^{2+} and Mg^{2+}), immersed in the PBS solution balanced with HEPES solution to maintain PH in the range 7.2–7.4, and placed under the AFM cantilever tip. Both cells and the AFM cantilever were observed with the microscope of the AFM. The cantilever tip can be positioned at any site of interest on cells using the Nanoscope controller software (Nanoscope 7.30, Veeco). Topography was imaged by contact mode with low deflection set point and scan rate such as 0.3 Hz to minimize the disturbance during the scan process.

2.3. Analysis of F–D curves and force volume method

Several different mechanical models have been developed for the analysis of the *F–D* curves. The classical Hertz model (Hertz, 1881) is expressed as

$$F = \frac{4\sqrt{R_C}}{3}\frac{E}{1-\nu^2}\delta^{3/2} \quad (1)$$

where δ, ν, R_C, and E indicate the indentation depth, the Poisson's ratio, the tip radius of the cantilever, and the elastic modulus. The Poisson's ratio was assumed to be 0.5 which is a typical value for soft incompressible material (Matzke et al., 2001). The classical Hertz model describing the indentation with force F into a homogeneous, soft, and semi-infinite elastic material is often applied in biological samples. Compared to the size of the tip of AFM cantilevers, a cell can be seen as a very large surface. However, an important assumption in the Hertz model is that the indentation depth should be less than the radius of the tip. In most cell indentation experiments, the indentation depth is up to hundreds nanometers which is much larger than the tip radius ~20 nm in our work.

Therefore, Sneddon model (Sneddon, 1965) is used in this study for elasticity calculation. This model assumes a rigid cone indenting a soft flat surface. The load and indentation depth relation is given by

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

where α is the half opening angle of the conical tip. Through controlling the indenting rate, the viscous effect of cells could be largely eliminated (A-Hassan et al., 1998). Fig. 1 shows a typical *F–D* curves. The Young's modulus is 12.35 kPa (with 95% confidence bounds) fitted by the Sneddon model, while the Hertz model has underestimated the value as 11.2 kPa.

To obtain the overall elasticity map of a cell, force volume measurements were performed on HAECs. The cells were scanned first to obtain a topography image to find the area of interest. Then a single *F–D* curve was generated from the ramp plot, which was used to determine the relative 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 were characterized as force volume imaging. Force volume (FV) measurement was performed to simultaneously obtain a height mapping image and matrix of force–

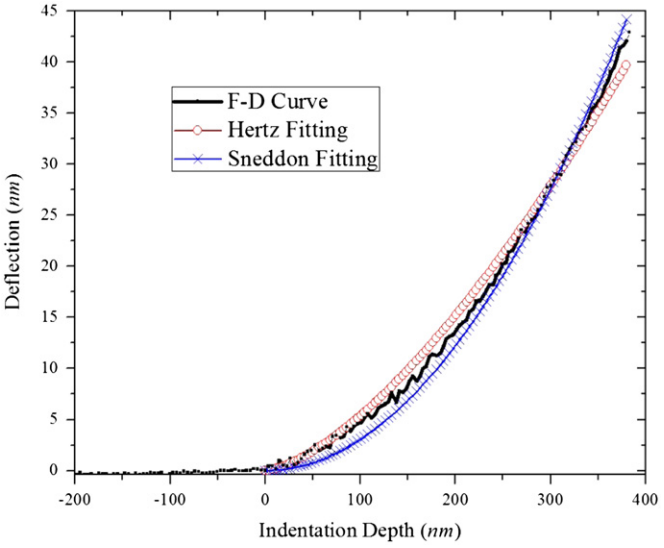

Fig. 1. A typical cantilever deflection versus indentation depth curve (black line without symbols) taken at a random site on the cell. Young's modulus fitted by Sneddon model (blue cross symbols) is 12.35 kPa, and by Hertz model (red circle symbol) is 11.2 kPa. The spring constant of the cantilever is 0.021 N/m. (For interpretation of the reference to color in this figure legend the reader is referred to the web version of this article.)