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Nanoscale characterization of dynamic cellular viscoelasticity by atomic force microscopy with varying measurement parameters

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ABSTRACT

Cell mechanics plays an important role in regulating the physiological activities of cells. The advent of atomic force microscopy (AFM) provides a novel powerful instrument for quantifying the mechanics of single cells at the nanoscale. The applications of AFM in single-cell mechanical assays in the past decade have significantly contributed to the field of cell and molecular biology. However, current AFM-based cellular mechanical studies are commonly carried out with fixed measurement parameters, which provides limited information about the dynamic cellular mechanical behaviors in response to the variable external stimuli. In this work, we utilized AFM to investigate cellular viscoelasticity (portrayed as relaxation time) with varying measurement parameters, including ramp rate and surface dwell time, on both cell lines and primary cells. The experimental results show that the obtained cellular relaxation times are remarkably dependent on the parameter surface dwell time and ramp rate during measurements. Besides, the dependencies to the measurement parameters are variable for different types of cells, which can be potentially used to indicate cell states. The research improves our understanding of single-cell dynamic rheology and provides a novel idea for discriminating different types of cells by AFM-based cellular viscoelastic assays with varying measurement parameters.

1. Introduction

Researches in the biophysical and biomechanical communities (Suresh, 2007) in the past decade have significantly shown that cell mechanics (e.g., elasticity (Moeendarbary et al., 2013), rheological property (Kuimova et al., 2009), and membrane tension (Diz-Munoz et al., 2013)) is closely related to the fulfillment of cellular physiological processes. Cellular rheological property is an important mechanical parameter. Cellular rheological property reflects cellular physiological behaviors, such as the motility of cytoplasm and the reorganization of actin cytoskeletons (Ehrlicher et al., 2015). Cellular rheological behaviors are connected to long-standing and unsolved fundamental questions in cell mechanics (Zhou et al., 2013). Cells dynamically alter their mechanical properties in the life. On the one hand, cells become solid to act as building blocks to keep the mechanical integrity of living organisms, whereas on the other hand, cells do change their mechanical properties to a more fluid-like behavior when it comes to migration and force generation (Ahmed and Betz, 2015). For example, single-cell mechanical assays have shown that the rheology of cells is related to the processes of cancer progression (Ketene et al., 2012). During cell mitosis, the spindle is maintained at the cell center by a force-generating machinery (Garzon-Coral et al., 2016), which results in the significant changes of cellular viscoelasticity (Pietuch and Janshoff, 2013). Hence, investigating cellular viscoelasticic properties is of fundamental significance for understanding the underlying mechanisms which guide cellular physiological behaviors.

Cellular viscoelasticity can be measured by passive microrheology or active microrheology (Fabry et al., 2001; Bausch and Kroy, 2006; Squires and Mason, 2010). The advent of atomic force microscopy (AFM) provides a novel method for actively characterizing the viscoelasticity of single cells at the nanoscale (Rigato et al., 2017; Li et al., 2017a). AFM uses a sharp tip (the tip radius is several nanometers (Dufrene et al., 2017)) mounted at the end of a micro cantilever to indent the cells, which senses the mechanical properties of cells, including elasticity (Radmacher, 2007; Kirmizis and Logothetidis, 2010; Lekka, 2016; Gavara, 2017), viscoelasticity (Hecht et al., 2015; Li et al., 2016; Rianna and Radmacher, 2017; Bruckner et al., 2017; Nematbakhsh et al., 2017), adhesion force (Dufrene, 2015), cell mass (Martinez-Martin et al., 2017), and molecular binding forces (Li et al., 2017b). AFM has been used alone (Lulevich et al., 2006) or combined

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with other techniques, such as computational method (Tartibi et al., 2015) and optical trapping (Nawaz et al., 2012), to characterize cellular viscoelasticity. Recently peak-force modulation AFM has been used to simultaneously investigate cellular viscoelasticity and cellular cytoskeleton organizations (Calzado-Martin et al., 2016; Schierbaum et al., 2017). However, it should be noted that current studies about AFM-based single-cell viscoelastic assays are commonly performed at fixed measurement parameters, which provide limited information about cellular rheological behaviors in response to variable external stimuli. Besides, whether cellular viscoelastic properties can be used to discriminate different types of cells has been still poorly investigated (Lekka, 2016). In this work, we utilized AFM to investigate cellular viscoelasticity on both cell lines and primary cells with varying measurement parameters (ramp rate, surface dwell time). The experimental results significantly showed the variable dependencies of different types of cells to the measurement parameters, which can be potentially used to discern different types of cells.

2. Materials and methods

2.1. Cell culture and agents

HEK-293 (human embryonic kidney cell line), MCF-7 (human noninvasive breast cancer cell line), MDA-MB-231 (human invasive breast cancer cell line) and Raji cells (human B-cell lymphoma cell line) were purchased from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). Cell culture medium and phosphate buffered saline (PBS) were purchased from Hyclone Laboratories (Logan, UT, USA). HEK-293 cells were cultured in DMEM medium (high glucose) containing 10% fetal bovine serum and 1% penicillin-streptomycin solution at 37 °C (5% CO_2 and 95% air). MCF-7 and Raji cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum and 1% penicillin-streptomycin solution at 37 °C (5% CO_2 and 95% air). MDA-MB-231 cells were cultured in Leibovitz L-15 medium containing 10% fetal bovine serum and 1% penicillin-streptomycin solution at 37 °C (5% CO_2 and 95% air). HEK-293, MCF-7, and MDA-MB-231 cells were directly cultured in Petri dishes. Raji cells were cultured in flasks. Poly-L-lysine, paraformaldehyde, and human peripheral blood mononuclear cell (PBMC) isolation solution were purchased from Solarbio Company (Beijing, China). Primary B lymphocyte isolation toolkits, including anti-CD19 antibody-labeled magnetic beads, MiniMACS magnetic separator, separation column, and running buffer, were purchased from Miltenyi Company (Auburn, CA, USA).

2.2. Primary B lymphocyte isolation

Primary B lymphocytes were isolated from the bone marrow and peripheral blood of a diffuse large B-cell lymphoma patient according to the established procedure (Li et al., 2016). The blood sample was prepared by the medical personnel from the Liaoning Cancer Hospital (Shenyang, China). The study was approved by the ethical committee of Liaoning Cancer Hospital. First, PBMCs were obtained from the anticoagulated blood (heparin treated) by density gradient centrifugation with the use of PBMC isolation solution. Second, the isolated PBMCs were incubated with anti-CD19 antibody-labeled magnetic beads for 15 min in the refrigerator (4 °C). After the incubation, the mixture was washed by PBS to remove the unbound magnetic beads. Subsequently, the cells were resuspended in 500 μL of PBS and then added into the isolation column. The column was placed in the magnetic field of MiniMACS separator. Running buffer was added into the column for three times (each time 500 μL was added) to remove the unlabeled cells. The column was then removed from the separator. After adding 1 mL running buffer into the column, the plunger was pushed into the column to flush out the labeled B lymphocytes. The collected B lymphocytes were immediately used for AFM experiments.

2.3. Atomic force microscopy

AFM experiments were carried out with the Bioscope Catalyst AFM (Bruker, Santa Barbara, CA, USA) which was set on an inverted microscope (Ti, Nikon, Tokyo, Japan). Four new AFM probes were used for the experiments. The nominal spring constant of the probe cantilever was 0.01 N/m. The material of the tip was silicon nitride and the nominal radius of the tip was about 20 nm. Before each experiments, the deflection sensitivity (nm/v) of the cantilever was calibrated by recording a force curve on the stiff substrate and then the exact spring constant was calibrated by applying the thermal noise module of the AFM. After each experiments, the AFM probes were washed by pure water. Living HEK-293 and MCF-7 cells were directly scanned by AFM at contact mode in the culture medium. The scan rate was 0.3 Hz. The scan line and sampling points for each scan line was 256. For imaging Raji cells and primary B lymphocytes, the living cells (Raji and primary B lymphocytes) were dropped onto the poly-L-lysine-coated glass slides, and then the glass slides were placed in a Petri dish containing 4% paraformaldehyde for 30 min. The cells were then washed by PBS for three times and subsequently AFM images were recorded in PBS at contact mode.

Cellular rheological properties were measured by controlling AFM tip to indent the living cells. Living HEK-293, MCF-7, and MDA-MB-231 cells grown in the Petri dishes were directly probed by AFM in the culture medium. Living Raji cells and primary B lymphocytes were dropped on the poly-L-lysine-coated glass slides. The glass slides were placed in the Petri dishes containing PBS and then cells (Raji cells and primary B lymphocytes) were probed by AFM. In order to eliminate the influence of detection positions on the measurements, all viscoelastic experiments are performed at the central areas of cells. Under the guidance of optical microscopy, the AFM probe is moved to the targeted cells. After the AFM probe contacts the cells, imaging mode is changed to force ramp mode by manual operations via the AFM manipulation Nanoscope Software (Bruker, Santa Barbara, CA, USA). Force ramp is performed at absolute trigger mode. The trigger threshold defines the maximal force applied on the sample. During the ramp mode, the AFM probe is controlled to firstly approach the cells with a certain rate (ramp rate). After the AFM probe achieves the maximal loading force preset in advance, the probe resides on the cell for a period of time (this is achieved by defining the surface dwell time). The probe then retracts from the cell. The illustrational description of the approach-reside-retract process is shown in Fig. 1A. During the approach-reside-retract process, the experimental stress relaxation curves of cells are recorded by an oscilloscope (LeCroy, New York, USA) which is linked to the AFM. During the experiments, we recorded stress relaxation curves with varying measurement parameters, including ramp rate (0.5 Hz, 1 Hz, 2 Hz, 4 Hz) and surface dwell (0.5 s, 1 s, 2 s, 4 s). For each types of cells (HEK-293, MCF-7, MDA-MB-231, Raji, and primary B lymphocyte), experimental stress relaxation curves were recorded on 20 cells.

2.4. Data analysis

The rheological parameters of cells can be extracted by fitting the experimental stress relaxation curves with adequate models, such as power-law model (Fabry et al., 2001; Rigato et al., 2017) and Maxwell spring-dashpot model (Li et al., 2018; Rianna and Radmacher, 2017). Here, two-order Maxwell spring-dashpot model was used to extract cell relaxation times from the experimental curves. The formula of two-order Maxwell spring-dashpot model (Li et al., 2017a) is:

$$F(t) = A_0 + A_i \sum_{i=1}^{2} e^{-t/\tau_i} \tag{1}$$

$$\tau_i = \frac{\eta_i}{E_i},\ i = 1, 2 \tag{2}$$

where F is the applied loading force of the AFM probe, A_0 is the