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Atomic force microscopy study revealed velocity-dependence and nonlinearity of nanoscale poroelasticity of eukaryotic cells

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ABSTRACT

Intracellular network deformation of the cell plays an important role in cellular shape formation. Recent studies suggest that cell reshaping and deformation due to external forces involve cellular volume, pore size, elasticity, and intracellular filaments polymerization degree change. This cell behavior can be described by poroelastic models due to the porous structure of the cytoplasm. In this study, the nanoscale poroelasticity of human mammary basal/claudin low carcinoma cell (MDA-MB-231) was investigated using indentation-based atomic force microscopy. The effects of cell deformation (i.e., indentation) velocity and depth on the poroelasticity of MDA-MB-231 cells were studied. Specifically, the cell poroelastic behavior (i.e., the diffusion coefficient) was quantified at different indenting velocities (0.2, 2, 10, 20, 100, 200 μm/s) and indentation depths (635, 965, and 1313 nm) by fitting the force-relaxation curves using a poroelastic model. Cell treated with cytoskeleton inhibitors (latrunculin B, blebbistatin, and nocodazole) were measured to investigate the effect of the cytoskeletal components on the cell poroelasticity. It was found that in general the MDA-MB-231 cells behaved less poroelastic (i.e., with lower diffusion coefficient) at higher indenting velocities due to the local stiffening up and dramatic pore size reduction caused by faster force load, and the cytoplasm is nonlinear in terms of poroelasticity. The poroelastic relaxation was more pronounced when the local cytoplasm porous structure was stretched by higher indentation. Furthermore, inhibition of cytoskeletal components resulted in pronounced poroelastic relaxation when compared with the control, and affected the nonlinearity of cell poroelasticity at different depth range inside of the cell. The comparison between the diffusion coefficient variation and the Young's modulus change under each indentation/treatment condition suggested that the cytoplasm porous geometry is more dominant than the cell Young's modulus in terms of affecting cell poroelasticity.

1. Introduction

Nowadays, attention toward cell rheology is growing due to the sensitivity of cell shape and deformation to external and internal biomechanical stimulation. For example, internal induced-forces due to biochemical interaction, intracellular organelle transport (Niclas et al., 1996), transcriptional change of genes (Coller et al., 2000), and signaling pathways (Elledge, 1996) proceed to elongation of the cells and cell cycling. Mutation of the genes, adapted pathways, and chemical interactions in different cell lines such as cancerous cells lead to significant cell rheological behavior change (Moeendarbary and Harris, 2014; Maloney et al., 2010; Sun et al., 2012; Brandao et al., 2003). Moreover, integrin-mediated focal adhesion (Balaban et al., 2001; Plotnikov et al., 2012), ion channels (Hayakawa et al., 2008), and cytoskeleton of the cell (Mitrossilis et al., 2009; Colombelli et al., 2009; Hayakawa et al., 2011) are responsive to extracellular forces applied on the cell. As cytoplasm forms the largest part of a cell by volume, its biomechanical property plays a key role in cell rheology by dictating the cell deformation magnitude and cell shape change rate. Therefore, investigating the biomechanical behavior of the cytoplasm is crucial in achieving in-depth understanding of cell rheology. Furthermore, as it is widely found that living cells probe, react, and adapt to external mechanical stimulation (Moeendarbary et al., 2013; Schillers et al., 2010), studying the mechanical properties of cytoplasm also promotes the modeling and quantification of the transduction of external mechanical stimulation into intracellular mechanical changes (Zhu et al., 2016; Charras et al., 2005, 2009).

Classical mechanical models have been implemented to biomechanics investigation of cell cytoplasm. The cortical shellliquid core models (e.g., the Newtonian liquid drop model (Tran-Son-Tay et al., 1991; Yeung and Evans, 1989), the compound Newtonian liquid drop model (Dong et al., 1990; Hochmuth et al., 1993), the shear thinning liquid drop model (Drury and Dembo, 1999, 2001), and the Maxwell liquid drop model (Sung and Schmid-Schb, 1988)) were developed to

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describe the rheology of cytoplasm in micropipette aspiration by assuming the homogeneity of the cell layer structure (Lim et al., 2006; Evans and Kukan, 1984; Yeung and Evans, 1989). To study the mechanics of cytoskeleton, solid models, such as the Hertzian model and the Sneddon model can be used to describe the contact mechanics between an elastic indenter and living cells by assuming the latter as an elastic isotropic body, and the contact is purely repulsive (Liu, 2006; Butt et al., 2005; Ghaednia et al., 2015a, 2016; Jackson et al., 2015). Due to the existence of attractive forces (e.g, van der Waals forces) when the indenters are brought into close proximity with the cells, the Johnson-Kendall-Roberts (JKR) (Chu et al., 2005) and the Derjaguin-Muller-Toporov (DMT) (Gao and Yao, 2004) models were then used to incorporate the effect of adhesion in Hertzian contact by taking the thermodynamic work of adhesion into account (Johnson et al., 1971; Derjaguin et al., 1975; Wu, 1982). The power-law structural damping model (Hildebrandt, 1969; Fredberg and Stamenovic, 1989; Maksym et al., 2000; Fabry et al., 2001; Kardel et al., 2017; Ghaednia et al., 2015b) was used for studying the viscoelasticity and the dynamic behavior of adherent cells (Ren et al., 2013). However, these models are not adequate enough to describe the biomechanical behavior of both the liquid flow (e.g., the cytosol) and the viscoelastic network (e.g., the cytoskeleton) —the biphasic nature of the cytoplasm. Therefore, a poroelastic model was implemented to study the biomechanics of cytoplasm, in which the cytoplasm was considered as a biphasic material consisting of a porous elastic solid meshwork (cytoskeleton, organelles, macromolecules) bathed in an interstitial fluid (cytosol) (Oster, 1989; Gu et al., 1997; Bachrach et al., 1995; Guilak and Mow, 2000; Moeendarbary et al., 2013). In the poroelastic model, the response of cells to external force load depends only on the poroelastic diffusion coefficient, D, which is determined by E the elastic modulus, ξ the pore size of the cytoskeleton meshwork, and μ the viscosity of the cytosol (Moeendarbary et al., 2013; Charras et al., 2009, 2008). According to the coarse graining of the physical parameters in the poroelastic model, cellular rheology results from the effects of the interstitial fluid (Keren et al., 2009), the related cell volume changes (Moeendarbary et al., 2013; CHENG), macromolecular crowding and the cytoskeletal network (Moeendarbary et al., 2013; Schillers et al., 2010), this is consistent with the observed rheological properties of the cell that the internal cell pressure equilibrates by redistribution of intracellular fluids in response to localized deformation (Charras et al., 2005, 2009; Keren et al., 2009; Rosenbluth et al., 2008; Zicha et al., 2003).

Poroelasticity studies of eukaryotic cells have been performed on atomic force microscopy (AFM) because of AFM's unique capability of applying force stimuli and then, measuring the sample response at specific locations in a physiologically friendly environment with piconewton force and nanometer spatial resolutions (Giridharagopal et al., 2012; Yan et al., 2017; Efremov et al., 2017). Weafer et al. (2015) investigated the force generation of the cells under an applied constant cyclic loading and unloading nominal strain rate at a frequency of 1 Hz, and it was found that the compression force was recovered and reached equilibrium at end of last cycle. Weafer et al. (2015). Hu et al. (2010) reported that interaction force between the AFM tip and the hydrogels was decreased during relaxation of the tip on the sample which led to deformation of the hydrogels (Hu et al., 2010). Tavakoli Nia et al. (2011) noted the poroelastic behavior of cartilage during relaxation experiment using AFM (Nia et al., 2011). It has been noted that the mechanical response of fluid-filled materials, like cells, depends on the time and length scales of the measurements and the mechanical deformation of the materials changes during the entire experimental time span (Kalcioglu et al., 2012). Moeendarbary et al. (2013) investigated the poroelastic behavior of the cell using micro bead when the approach velocity was 10 μm/s, and it was found that the components of the cells including actin, microtubules, myosin, and intermediate filaments affect the diffusion coefficient of the cell (Moeendarbary et al., 2013). However, since the cytoplasm of a living cell is highly heterogeneous and consists of a multi-layer structured viscoelastic cytoskeleton (i.e., velocity dependent), the cytoplasm poroelasticity quantified in previous work was limited to the specific measurement specifications and physical conditions (e.g., indenter size, approach velocity, and indentation depth). Particularly, due to the biphasic nature of living cells, the cell deformation rate (i.e., the AFM probe approach velocity) affects the measured cell stiffness significantly (Moeendarbary et al., 2013; CHENG), and the deformation/indentation depth range determined the layers of the cells triggered and measured during the mechanical quantification (Kasas et al., 2005; Fuhrmann et al., 2011). Thus, to achieve in-depth understanding of the cell rheological behavior, study the poroelastic behavior of cytoplasm under different external excitation conditions is necessary.

In this study, we investigated the contribution of external force conditions to cellular rheology of human mammary basal/claudin low carcinoma cell at nanometer scale using AFM. Specifically, the cells were probed under forces with different approach velocities and magnitudes, and the poroelasticity diffusion coefficient was then quantified for each condition by fitting the force-relaxation curve using an empirical poroelastic model. Furthermore, to study the effect of internal cell structural property on determining the cell rheology and the nonlinearity of cell poroelasticity, we examined the importance of cytoskeleton in affecting cell poroelasticity.

2. Materials and methods

2.1. Chemicals

The human mammary basel/claudin low carcinoma cell line (MDA-MB-231) and Leibovitz's L-15 Medium (L-15) were purchased from American Type Culture Collection (ATCC, Rockville, MD, USA). Dubecco's Modified Eagles Medium (DMEM) and Dimethyl sulfoxide (DMSO) were purchased from Sigma Aldrich (St. Louis, MO, USA). Fetal bovine serum and penicillin-streptomycin were obtained from Gibco (Grand Island, New York, USA). Latrunculin B and blebbistatin were purchased from Millipore sigma (Billerica, Massachusetts, USA). Nocodazole was purchased from Acros organics (New Jersey, USA).

2.2. Cell culture and treatment

MDA-MB-231 cells were cultured in the following cell growth medium: DMEM containing 10% fetal bovine serum (FBS) and 1% penicillin-steptomycin (pen-strep). The cells were subcultured at a density of 2.0×10^4 cells/ml on 35 mm cell culture dishes (Falcon, Durham, NC, USA) and maintained at 37 ° C in 5% CO_2 incubator 24 h prior to the AFM measurement. For the AFM poroelasticity measurements, the existing medium in the dishes was replaced by L-15 with the same concentration of FBS and pen-strep to remove dead and loosely attached cells, and to maintain the health of the cells during the experiment.

2.3. Cytoskeleton treatments

To investigate the contribution of cytoskeleton components on cell poroelasticity, the cells were treated with latrunculin B (750 nM to depolymerize F-actin), nocodazole (5 μ M to depolymerize microtubules), and blebbistatin (100 μM to inhibit myosin II ATPase) separately in the aforementioned cell growth medium and incubated 30 min prior to the AFM measurements (Moeendarbary et al., 2013). Then, the cell growth medium was replaced by L-15 with 10% FBS, 1% pen-strep, and the same drug concentration such that the drug effect was present during all measurements. Stock solutions were made by dissolving each drug in DMSO. Then, the aforementioned stock concentrations were prepared by adding the medium dropwise into the solution (Mikulich et al., 2012). The DMSO concentration during the treatments and AFM measurement was 0.05%. To study the effects of the treatments on cell poroelasticity, the untreated cells were exposed to the same DMSO concentration, and used as control.