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Microenvironment and cell mechanics

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Abstract: Microenvironment contains biophysical and biochemical elements to maintain survival, growth, proliferation, and differentiation of cells. Any change can lead to cell response to the mechanical forces, which can be described by elasticity. It is an indicator of a cell's state since it plays an important role in many cellular processes. In many cases, cell elasticity is measured by using discontinuous manner, which may not allow elucidating real-time activity of individual live cells in physiological condition or cell response against microenvironmental changes. I argue that measuring cell elasticity using continuously repetitive nanoindentation technique is important that should be considered. As an example, I discuss mechanics of human embryonic kidney (HEK) cells in various conditions. In resting cells, there is an activity of the cytoskeleton whose oscillation amplitude is strongly affected by the intracellular calcium, and the collective activity of myosin motor proteins induces elasticity oscillation. Experimental results also reveal that actin cytoskeleton and cell membrane determine cell mechanics.

Introduction

Cell mechanics is a factor that controls important cellular functions, including cell polarization, migration, growth, and proliferation, as well as trafficking inside the cytoplasm and organization of organelles (Wu *et al.*, 2018). It defines response of cells to the mechanical forces exercised by the surrounding microenvironment, including the extracellular matrix and other cells. These forces exert continuously extensional, compressive, and shear forces on cells *in vivo* (Lautenschlager *et al.*, 2009). The deformability of cells in response to mechanical forces plays an important role in the homeostasis of adult organs and tissues and the development of proper embryonic. The ability to transport intracellular cargo, to resist deformation, and to change shape during movement depends on the cytoskeleton, which is an interconnected network of a variety of regulatory cytoplasmic proteins, e.g., myosin motors (Wu *et al.*, 2018) or filamentous polymers including microtubules and actin filaments. Cytoskeleton acts as biochemical and physical interface for various cellular processes, involves in downstream and upstream in a large variety of signaling pathways, and determines elasticity and local behavior of cells (Fletcher and Mullins, 2010; Schillers *et al.*, 2010).

With the development of nanoindentation technique based atomic force microscopy (AFM), cell elasticity can be easily measured using discontinuous manner. This allowed discriminating normal from cancerous cells (Lekka *et al.*, 1999; Cross *et al.*, 2007), distinguishing either cells from other materials (Weisenhorn *et al.*, 1993) or gene-deficient cells from intact ones (Goldmann *et al.*, 1998), and detecting changes in plasma membrane composition (Bui and Nguyen, 2016) or tension (Herant *et al.*, 2005). Although lot of work has been carried out to characterize cell elasticity, the origin of the elasticity fluctuation of individual cells remains unclear. Recent studies introduced an additional approach using repetitive indentation of cells by an AFM probe, which allows following the activities of cell elasticity of individual live cells. Here I suggest that this approach should be considered since it allows real-time monitoring of the dynamics of cell elasticity and cytoskeletal as well as membrane activities with a high time resolution.

Cell Elasticity in Physiological Condition

Cell elasticity, an integrative parameter reflecting cell mechanics, is not a constant in standard physiological condition. It is oscillating and the oscillations are spontaneous (self-induced) (Placais *et al.*, 2009; Sanyour *et al.*, 2018). The magnitude of the elasticity oscillations is different from cell to cell, cell type, or cell state and can reach up to several times (Bui and Nguyen, 2020; Schillers *et al.*, 2010). These oscillations are generally powered by the collectively working within assemblies of the molecular motors (Julicher and Prost, 1995; Placais *et al.*, 2009) and

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are related to various functions, such as contraction, cell length oscillation, and movement of cell organelles (Schillers *et al.*, 2010). They may be potentially caused by the intracellular free calcium oscillations, which play a pivotal role in fundamental cell signaling processes (Atri *et al.*, 1993; Berridge *et al.*, 2003; Uhlen and Fritz, 2010). The activity of myosin motors dependent upon calcium is one of these processes. These motors participate in the organization of the cytoskeleton, which enables cells to sense their environment and to contract (Woolner and Bement, 2009), and plays an important role in polarity formation, cell migration, and cytokinesis (Sellers, 2000). They also regulate downstream biochemical signaling pathways through phosphorylation and dephosphorylation of the light chain (Watanabe *et al.*, 2007). Especially, they actively affect the elasticity of the cytoskeleton (Koenderink *et al.*, 2009; Martens and Radmacher, 2008; Sweeney and Houdusse, 2010), whose function is to connect the cell biochemically and biophysically to the external environment, maintain and arrange the integrity of intracellular compartments, and generate forces that enable the cell to change shape and move. The cytoskeleton can offer these various functions since it is an adaptive and a dynamic system, not a static structure. Its components including regulatory motor proteins and biopolymers are oscillating and cytoskeleton's state primarily determines cell elasticity (Fletcher and Mullins, 2010), one of the most important mechanical properties of cells which are often connected to their function and state.

In complex cell systems, the simplest case of dynamic processes is oscillations whose emergence is subtle because of its dependence on the dynamic properties of the collective behaviors and interacting components. This means that the emergence of oscillations is common in cell biology (Kruse and Julicher, 2005). These spontaneous oscillations can be easily observed by applying repetitive indentation of cells by an AFM tip to measure the cell elasticity. This approach allows us to follow dynamics of the elasticity and subsequently of the cytoskeletal activity of individual live cells with high time resolution (Bui and Nguyen, 2018, 2020). However, this approach was not applied in most of studies on the cell mechanics leading to a false positive (overestimated) or false negative (underestimated) value of cell elasticity. For example, if the nanoindentation measurement is carried out in less than two minutes on an individual cell as commonly reported, the elasticity of that cell, in case a spherical AFM probe is used with an applied force of 200 pN, can be from 150 Pa to 400 Pa as shown in Fig. 1 depending on the time point of the measurement (Bui and Nguyen, 2020). This may also be a factor that contributes to the variation in the reported values of the cell elasticity (Wu *et al.*, 2018).

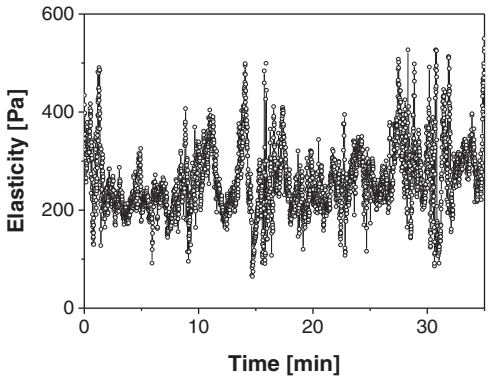

FIGURE 1. Real-time monitoring of elasticity of an individual live HEK cell in its standard physiological living microenvironment reveals an elasticity fluctuation. Each data point is the result obtained from a single force-indentation cycle. Adapted from Bui and Nguyen (2020).

Cell Elasticity in Perturbed Condition

Cell elasticity is defined not only by intracellular processes in standard condition, but also by microenvironmental factors like biophysical and biochemical surrounding in perturbed condition. Therefore, it can serve as an integrative parameter to characterize cell states and represent a variable of life. In physiological condition, variabilities of biophysical and biochemical processes result in a certain elasticity range while in perturbed condition, exogenous or endogenous variables can affect cell behavior and frequently induce changes in cell elasticity (Schillers, 2019). One of the factors that directly contributes to elasticity changes is motor protein myosins. It is proved that inhibiting crosslinking between actin filaments and nonmuscle myosin II by blebbistatin to disrupt the actin cytoskeleton induces a significant decrease in cell elasticity (Schiele *et al.*, 2015). Another factor is calcium ions, which play a crucial role in cytoskeleton organization (Ho *et al.*, 1999). Increasing or decreasing intracellular calcium concentration by ionomycin or BAPTA-AM (a membrane-permeable calcium chelator) leads to cell stiffening or softening. Actin cytoskeleton is also an important factor defining cell elasticity and when actin filaments are disaggregated by cytochalasin or latrunculin, a distinct decrease in the cell elasticity has been observed (Rotsch and Radmacher, 2000).

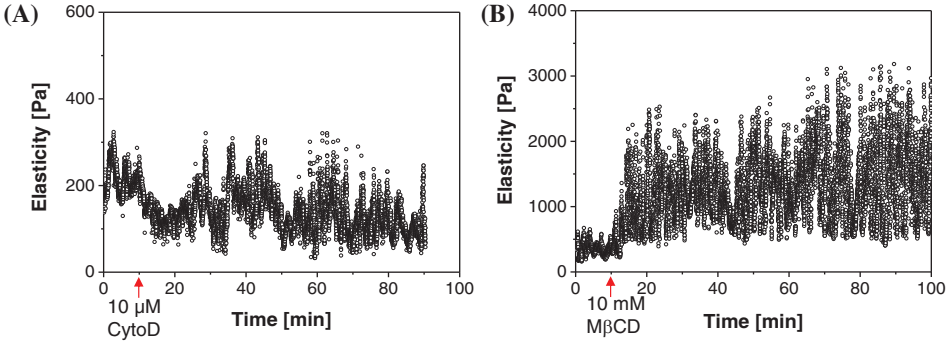

FIGURE 2. Time course of changes in elasticity of individual HEK cells while disrupting actin cytoskeleton by cytochalasin D (A) and depleting the membrane cholesterol by methyl-β-cyclodextrin (B). Arrows point to the time points the drugs were added during repetitive nanoindentation measurement. Adapted from Bui and Nguyen (2020).

Together with cytoskeleton, cell membrane also has a major impact on determining cell elasticity. It is the outermost layer that envelopes cell cytoplasm and plays a pivotal role in protecting the cell from extracellular microenvironment. It supports and maintains the cell shape and controls substances in and out of cells. Changes in cell membrane composition also affect cell elasticity (Bui and Nguyen, 2016; Byfield *et al.*, 2004). These changes can be easily obtained with a conventional nanoindentation technique. However, the dynamics of cytoskeleton and real-time response of cells against sudden changes in physiological environments can be observed only when a repetitive nanoindentation is applied as shown in Fig. 2 (Bui and Nguyen, 2020). Thus, this approach can serve as an add-in technique in the measurement of cell elasticity by AFM.

Conclusion

The technique of real-time monitoring of cell elasticity following time using repetitive nanoindentation of cells should be considered as a valuable complement for the conventional approach to evaluate the cell elasticity. This additional approach allows us to follow dynamics of the cytoskeletal activity, subtle changes of cell membrane, and subsequently dynamics of cell elasticity under various conditions with high time resolution. This discloses elasticity fluctuation in HEK cells which was driven by activity of Ca^{2+}-dependent motor proteins. This also shows that the collective activity of the motor proteins defines cell dynamics, reflecting changes of the biochemical and biophysical microenvironment. The cell elasticity reflects not only the status of the membrane and cytoskeleton, which are determined by both passive and active components, but also the system behaviour rather than the specific characteristic of individual components since the cytoskeleton's state is the result of a complex regulatory machinery. Thus, this time-resolved elasticity measurement approach provides deep insights into the activity of cell mechanics in response to changes of the physiological microenvironment and its underlying mechanisms. Eventually, applying this technique in combination with an appropriate setting of parameters, especially the applied force to the AFM cantilever and temperature of the living microenvironment of cells, for the measurement may help us to avoid false positive or false negative evaluation of cell elasticity.

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