Chapter 8

Dynamic Mechanical Response of Epithelial Cells to Epidermal Growth Factor

Jun Xi, Lynn S. Penn, Ning Xi, Jennifer Y. Chen and Ruiguo Yang

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/49977

1. Introduction

1.1. Mechanical properties of cells and their biological significances.

As a viscoelastic body, the cell exhibits both elastic and viscous characteristics (Kasza, 07). Although these mechanical properties have not been attributed wholly to a single element, such as the cytoskeletal network, the cytoplasm, the cell membrane, or the extracellular network (Janmey et al., 2007), it is agreed that they are determined predominantly by the cytoskeleton, a network of biopolymers in the form of actin filaments, microtubules, and intermediate filaments. The dynamic assembly and disassembly of these biopolymers give the cell the ability to move and to modulate its shape, elasticity, and mechanical strength in responses to mechanical and chemical stimuli from the external environment (Fletcher & Mullins, 2010). Among these cytoskeletal polymers, actin filaments are known to be primarily responsible for the rigidity of the cell. An increase in the concentration of actin filaments typically results in an increase in the rigidity of the cell, which can be characterized by Young's modulus (Satcher Jr & Dewey Jr, 1996).

The cytoskeleton is also essential in regulation of cell signaling and trafficking (Janmey, 1998; Papakonstanti & Stournaras, 2008). In particular, the structure of the cytoskeleton plays an essential role in EGFR signaling and trafficking that is initiated by the binding of epidermal growth factor (EGF) to the EGF receptor (EGFR) (Ridley, 1994; Song et al., 2008). EGF is a protein molecule known to play a crucial role in the regulation of cell growth, proliferation, differentiation and motility. EGFR is a transmembrane receptor that consists of an extracellular ligand-binding domain, a transmembrane domain, an intracellular tyrosine kinase domain, and a C-terminal regulatory domain (Scaltriti & Baselga, 2006). Binding of EGF to the extracellular domain of EGFR leads to the dimerization of EGFR, which in turn stimulates tyrosine kinase activity of the receptors and triggers autophosphorylation of

specific tyrosine residues within the cytoplasmic regulatory domain. The activation of tyrosine kinases initiates multiple downstream signaling pathways such as Ras/Raf-1/MAPK (Scaltriti & Baselga, 2006), PI3Kinase/Akt/mTOR (Ono & Kuwano, 2006), Src/NFKb (Lee C.-W. et al., 2007; Silva, 2004), catenin/cytoskeleton (Yasmeen et al., 2006) and PAK-1 /Rac pathways (McManus et al., 2000).

It is known that EGFR signaling induces drastic morphological changes, such as rounding of cells, induction of membrane ruffling and extension of filopodia (Bretscher, 1989; Chinkers et al., 1981). These changes can be attributed to the remodeling of cytoskeletal structures (Rijken et al., 1991), which may also alter mechanical properties of the cells (Kasza et al., 2007; Stamenovic, 2005). Currently, the connection between cell signalling and alterations of the mechanical properties of cells is still not fully understood in general. Information concerning the effects of EGF stimulation on the mechanical properties of cells will certainly provide insights into this connection. In addition, since EGFR is highly expressed in a variety of human tumors (Dei Tos & Ellis, 2005) and mutations in EGFR can produce aberrant cell signaling that often leads to uncontrolled cell growth and a malignant phenotype, such information will also shed light on the link between cell mechanical properties and human diseases (Bao & Suresh, 2003).

Many highly sensitive techniques have been developed over the years to assess mechanical properties of cells (Addae-Mensah & Wikswo, 2008). These include atomic force microscopy (Smith et al., 2005), magnetic twisting cytometry (Wang et al., 1993), micropipette aspiration (Alexopoulos et al., 2003), optical tweezers (Svoboda & Block, 1994), Shear-flow methods (Usami et al., 1993), particle-tracking microrheology (Wirtz, 2009), cantilever beams (Galbraith & Sheetz, 1997), and others. Each technique probes a cell or cells in a different manner and does not necessarily measure the same aspects of a cell as another technique. Thus, the use of more than one technique to study the same object (i.e., cell) may prove useful. This chapter describes the application of two sensitive techniques, the atomic force microscopy and the quartz crystal microbalance with dissipation monitoring, to the study of the mechanical properties of cells in response to exposure to EGF.

1.2. Probing mechanical response of cells with atomic force microscopy

Atomic force microscopy (AFM) is one of the most popular choices for probing the mechanical properties of cells, because individual cells can be probed in high sensitivity and resolution with a minimum of force (Radmacher Manfred, 2007). To measure the mechanical properties of the cell with AFM, the top surface of a live cell is indented with the sharp tip located at the end of a cantilever (a probe). The cantilever is mounted on a piezoelectric tube that moves the cantilever down and up in the vertical direction toward and away from the surface of the cell. The deflection in the cantilever is typically measured by a laser that tracks a spot on the tip of the cantilever. From the position of the cantilever and its deflection, force-displacement curves during the indentation of the cell by the probe are generated as shown in Figure 1 (Radmacher M., 1997).

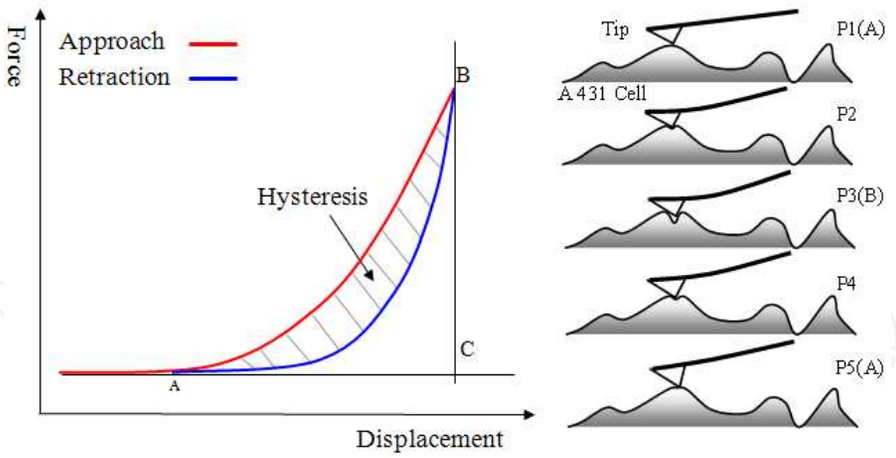

Figure 1. Typical force-displacement curves generated for the approach and retraction of the AFM probe. Approach and retraction correspond to mechanical loading and unloading of the AFM probe on the top surface of the cell.

To indent epithelial cells that are being treated with biologically active molecules, the force applied on the cells by the AFM probe is often kept approximately 50 nN or slightly lower to minimize the adverse effects on the cells caused by the probe. With the force at this level, the probe can have the probing depth that is sufficiently deep (100 to 500 nm) to register the cytoskeleton remodeling (Schillers et al., 2010) but is still shallow enough to avoid influence from the nucleus and the solid substrate on which the cells rest (Melzak et al., 2011). In addition to a low magnitude of loading, the velocity of loading should be kept low enough so that the transient friction interactions between the probe tip and the cell surface are avoided (Alcaraz et al., 2003).

Force-displacement curves acquired with all of these precautions in place then can be used to estimate values for Young's modulus and energy dissipation of the cell. The Young's modulus of a cell can be extracted from a curve of unloading force displacement with the aid of the Herzian elastic contact model for a conically shaped tip indenting an elastic body (Touhami et al., 2003):

$$F = kd = \frac{2}{\pi} \bullet \frac{E}{1 - \nu^2} \delta^2 \tan\alpha \tag{1}$$

where F is the applied force, d is the deflection of the cantilever, and k is the spring constant of the cantilever. Also, α is the half angle of the cone-shaped tip, υ is the Poisson ratio (taken to be 0.5, for an incompressible material), δ is the indentation depth, and E is Young's modulus. It should be noted that the force-displacement curves are dependent on the frequency of the probe (Hoffman & Crocker, 2009); this means that the estimated values of modulus are not unique.

In the estimation of the Young's modulus, the cell is assumed to be an elastic body, i.e., to return all of the energy deposited during the loading portion of the indentation process. However, in reality, the cell is not perfectly elastic but exhibits some dissipative behaviour.

This dissipative behavior is manifested as a loss (as heat to the surroundings) of some of the energy stored during loading, and can be seen in the indentation process as hysteresis in a cycle of force displacement (Figure 1). In a cell, energy dissipation is believed to be accomplished by internal friction and/or viscous damping mechanisms (Alcaraz et al., 2003; Smith et al., 2005). In AFM, the mechanical energy dissipated per cycle of indentation is given quantitatively by the area of the hysteresis loop enclosed by the approach and retraction curves (Alcaraz et al., 2003), as shown in Figure 1.

1.3. Probing mechanical response of cells with quartz crystal microbalance with dissipation monitoring

In contrast to AFM, the quartz crystal microbalance with dissipation monitoring (QCM-D) has not been widely used in characterization of cell mechanics. The QCM-D is an ultrasensitive piezoelectric device (Hook F. et al., 1998; Rodahl Michael et al., 1996; Rodahl M. & Kasemo, 1996) that is able to detect mass coupled (adsorbed or adhered) to the surface of the sensing element. The sensing element is a single piezoelectric quartz crystal in the form of a thin disc with a metal electrode deposited on its underside. This sensor crystal is set into free vibration in shear mode by means of a pulse of current. In air, the sensor crystal has a characteristic resonant frequency; this frequency is changed when any material, liquid or solid, is coupled or attached to it by adsorption or adhesion.

The mass of an ultrathin and elastic adsorbed layer, which exhibits negligible dissipation, is given by the Sauerbrey equation:

$$\Delta m = \frac{C}{n \times \Delta f} \tag{2}$$

where Δm is mass, C is the instrument sensitivity constant in ng/cm^2 of the crystal surface area, n is the frequency overtone number (n = 1, 3, 5, …), and Δf is the simplified representation of Δf_n, the change in resonant frequency at the overtone number n, caused by the attached mass (Sauerbrey, 1959). The acoustic shear wave from the vibrating sensor penetrates the attached layer without disrupting it and the instrument monitors Δf of the layer at multiple overtones (Hook Fredrik et al., 2001) as a function of time. The instrument simultaneously monitors the change in dissipation factor, ΔD, which is defined as the ratio of the dissipated energy to the stored (elastic) energy per vibrational cycle. An ultrathin, elastic layer exhibits a negligible value of ΔD, but a layer that is viscoelastic exhibits a non-negligible and measureable value of ΔD. Both Δf and ΔD provide information about the mechanical response of a layer attached to the surface of the sensor crystal, and any type of change in the layer produces changes in these quantities.

The QCM has been used to assess the changes in mass and mechanical properties of a layer of biomolecules immobilized on the surface of the quartz crystal (Dixon, 2008). Specific examples include the use of the QCM to show protein adsorption (Hook F. et al., 1998), to indicate changes in an immobilized layer caused by ligand–receptor interactions (Janshoff et

al., 1997; Lee H. et al., 2010), to detect nucleic acid hybridization (Furtado & Thompson, 1998), and to study immunoresponse (Aizawa et al., 2001).

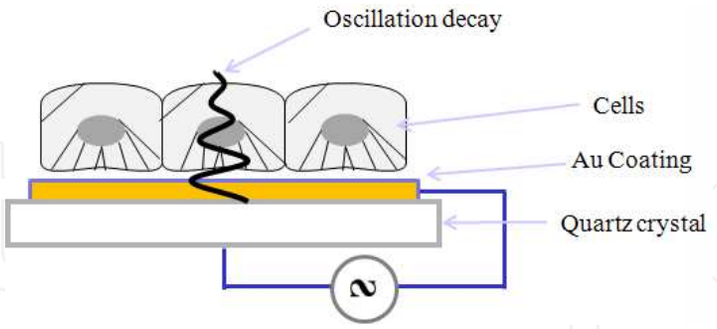

Figure 2. Diagram of a layer of cells attached to the surface of a sensor crystal in the QCM-D technique. The vibrational wave originating from the piezoelectric sensor crystal penetrates the cells from the bottom and diminishes with distance above the sensor surface. This figure is adapted with permission from (Chen et al., 2011). Copyright 2011, American Chemical Society.

In the field of cell biology, the QCM technique has become particularly attractive for its capability to study cells in a label-free manner (Heitmann et al., 2007; Janshoff et al., 1996; Matsuda et al., 1992; Redepenning et al., 1993; Wegener et al., 1998). Importantly, the technique is non-invasive to mammalian cells when the amplitude of shear oscillation is kept under 1 nm (Heitmann & Wegener, 2007). It has been used for determining the kinetics of cell attachment and spreading (Fredriksson et al., 1998; Nimeri et al., 1998) and for monitoring the long term growth of cells (Otto et al., 1999; Reipa et al., 2006). More recently, the QCM has been applied to characterization of cell viscoelasticity (Alessandrini et al., 2006; Galli Marxer et al., 2003; Li et al., 2008; Marx et al., 2005; Pax et al., 2005; Voinova et al., 2004). When the instrument used has the capability for monitoring the change in dissipation factor as well as frequency, the technique is termed QCM-D (QCM with dissipation monitoring). Because the acoustic signal diminishes exponentially with distance above the surface of the quartz crystal oscillator on which the cells are deposited, the QCM probes primarily the basal area of the cell monolayer (Heitmann et al., 2007; Le Guillou-Buffello et al., 2011). The test configuration is shown in Figure 2. Thus, Δf and ΔD, for adherent cells measured by the QCM-D technique can be expected to be related to mechanical changes in basal area of the cells and may be related to strength and quality of adhesion between the cell and the surface it contacts (Fredriksson et al., 1998; Rodahl M. et al., 1997).

2. Investigation of the dynamic mechanical response of an A431 cell monolayer in response to EGF

2.1. Mechanical behavior of an A431 cell monolayer probed with AFM

An example of the use of the AFM to assess change in the mechanical response of cells upon exposure to a biologically active molecule is illustrated in this section. Figure 3 shows the Young's moduli, obtained at a probe speed of 5.8 μm/s and indentation depth of ~500 nm, for two hundred randomly selected A431 cells before and after the treatment with a 40-nM

EGF solution (Yang et al., 2012). The focus is on the comparison, rather than on the modulus values themselves. The two samples show a statistically significant increase ($p < 0.05$) in average modulus as a result of the treatment: 11.2 ± 2.8 kPa for untreated cells and 18.7 ± 2.0 kPa for treated cells.

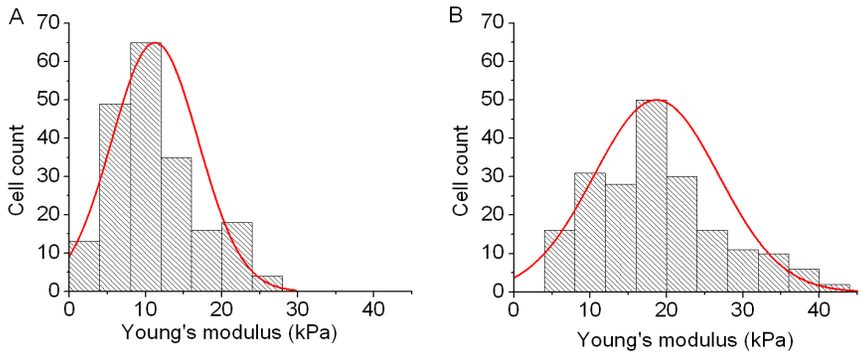

Figure 3. Histograms of the distributions of the Young's modulus of two hundred randomly selected A431 cells before (A) and after (B) the treatment with a 40-nM EGF solution in buffer (Yang et al., 2012).

Figure 4 shows changes in amount of the dissipated energy of cells upon EGF stimulation. It is evident that energy dissipation of a cell, as indicated by the area of the hysteresis loop, increases after the treatment with 40 nM EGF (Figures 4A and 4B). Figure 4C summarize the distribution of such differences exhibited by one hundred randomly selected cells. A statistically significant difference ($p < 0.05$) in energy dissipation per cycle is shown: 3.09 ± 0.79 fJ before the treatment and 5.10 ± 0.71 fJ after the treatment.

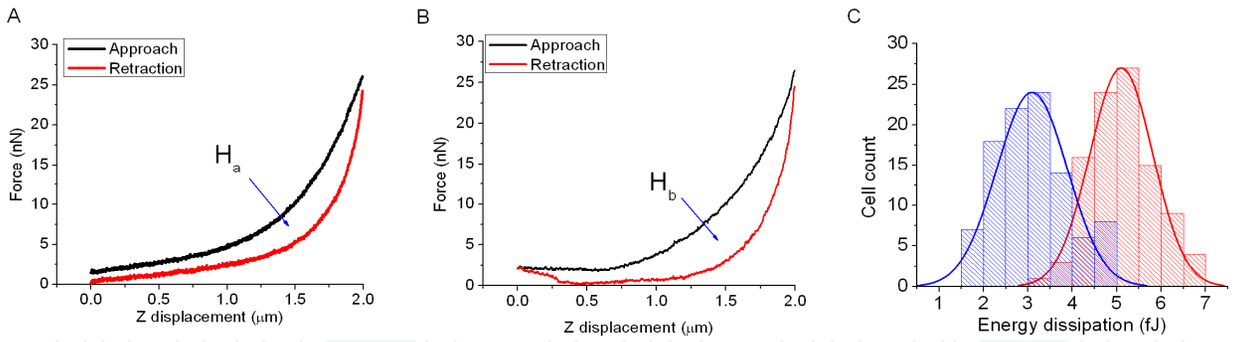

Figure 4. Force-displacement curves for a single cell before (A) and after (B) the treatment with 40 nM EGF. Histograms (C) of energy dissipation for one hundred randomly selected cells before (blue) and after (red) the treatment with EGF.

The underlying meaning of a simultaneous increase in both stiffness (Young's modulus) and energy dissipation of the cells upon the EGF treatment can be better understood with the soft glassy rheology (SGR) model (Fabry et al., 2001). In this model, the cell is considered as a soft glassy material that is structurally disordered and metastable (Sollich, 1998). The hysteresivity of the cell, η, defined as the fraction of the elastic (input) energy that is dissipated as heat, is frequency insensitive and remains constant in the low frequency regime (Fredberg & Stamenovic, 1989).

Although both stiffness and dissipation of the cells increase simultaneously, the hysteresivity of the cells, which is determined based on the ratio of area within the hysteresis loop to area under the approach curve (Figure 1) (Collinsworth et al., 2002; Fung, 1984; Smith et al., 2005), is not constant but increases as a function of time, shown in Figure 5. According to the SGR model, upon a non-thermal stimulation (e.g., ATP depletion, or cell relaxing agent), the cell can undergo a change in mechanical ordering state either toward the glass transition as hysteresivity decreases or away from the glass transition as hysteresivity increases. So the cell can modulate its mechanical state between a more solid-like state and a more liquid-like state (Smith et al., 2005). The increase in hysteresivity in Figure 5 implies that the mechanical state of the EGF-treated cells moves away from the glass transition and possibly takes on a more fluidic behavior. This interpretation is consistent with morphological changes observed in A431 cells responding to EGF stimulation, where the cells undergo cell rounding, membrane ruffling, and filopodia extension, all of which might be facilitated by a more fluid-like state of the cells (Chinkers et al., 1981).

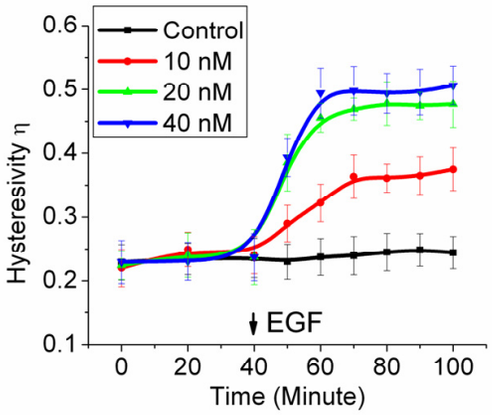

Figure 5. Hysteresivity versus time for exposure of A431 cells to EGF at 0, 10 nM, 20 nM, and 40 nM (Yang et al., 2012). A 30-min baseline was established for each measurement prior to the addition of EGF. Trend lines were used to connect each data point to illustrate the trend for each response curve.

2.2. Mechanical behavior of an A431 cell monolayer probed with QCM-D

An example of the use of QCM-D to assess change in the mechanical response of cells upon exposure to a biologically active molecule is shown in Figure 6. This figure shows Δf and ΔD obtained simultaneously in response to addition of a solution of EGF. The spike at 5 min is an artifact of the manual addition of the solution with a pipette. After the initial spike, ΔD goes down and then slowly recovers, while Δf steadily increases with time (Chen et al., 2011). Because ΔD was substantially more sensitive than Δf to dosage of EGF, we focus the descriptions primarily on the changes in ΔD.

Figure 7 shows the QCM-D measurement of ΔD for a monolayer of A431 cells exposed to buffer alone and to EGF. Figure 7A reveals that, after the spike caused by manual addition

of the solution, the control cells (0 nM EGF) showed a small decrease in Δ*D*, while the experimental cells (10 nM EGF) exhibited a large decrease. This difference represents the change in mechanical energy dissipation of the basal areas of the cells induced by EGF.

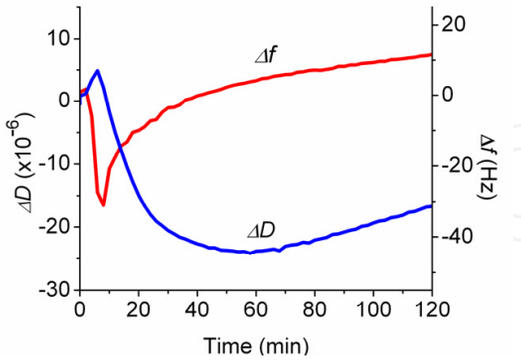

Figure 6. Typical response of A431 cells to EGF. Both Δ*f* and Δ*D* are shown as a function of time.

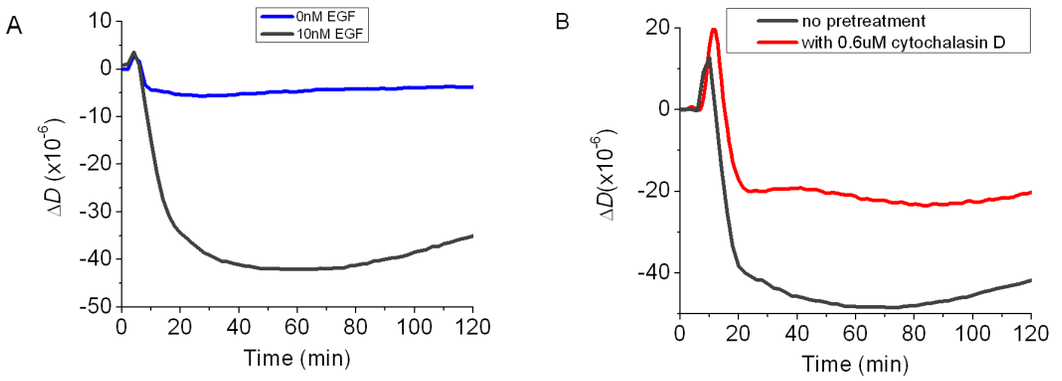

Figure 7. Real-time QCM-D measurements of the Δ*D* response of a monolayer of A431 cells to exposure to EGF (Chen et al., 2011). (A) Δ*D* response of the cells in the presence (bottom) and absence (top) of 10 nM EGF. (B) Δ*D* response of the cells pretreated with an inhibitor of actin polymerization, cytochalasin D, showing suppression of the EGF-induced response. Both figures are adapted with permission from (Chen et al., 2011). Copyright 2011, American Chemical Society.

A likely cause of the reduction in Δ*D* upon exposure to EGF was the remodeling of the cytoskeleton, a process integral to the mechanical response of the cell. This remodeling can be interfered with if the polymerization of actin is inhibited. Figure 7B shows that pretreatment of the monolayer of cells with a 0.6 μM solution of cytochalasin D, a potent, cell-permeable inhibitor of actin polymerization (Schliwa, 1982), diminished the magnitude of the response of the cells to EGF. This result supports the remodeling of the cytoskeleton as the cause of the Δ*D* response, which echoes the previous finding of Heitmann and coworkers' (Heitmann et al., 2007).

It is desirable to connect the EGF-induced changes in Δ*D* to EGFR-mediated cell signaling. As already mentioned, one of the domains of EGFR, the receptor for EGF, is the intracellular tyrosine kinase domain. This domain is integral to the signaling pathway within the cell that transmits the effects of EGF to other parts of the cell, and if this domain is inhibited, the signaling pathway is disrupted. PD158780 has been shown to be a potent inhibitor of the tyrosine kinase domain for EGFR (Rewcastle et al., 1996). With a pretreatment with this

inhibitor, the cells showed a greatly suppressed Δ*D* response upon exposure to EGF, while the cells without pretreatment showed the expected decrease in Δ*D* (Figure 8). These results are evidence that the EGF-induced the Δ*D* response is due to EGFR-mediated cell signaling.

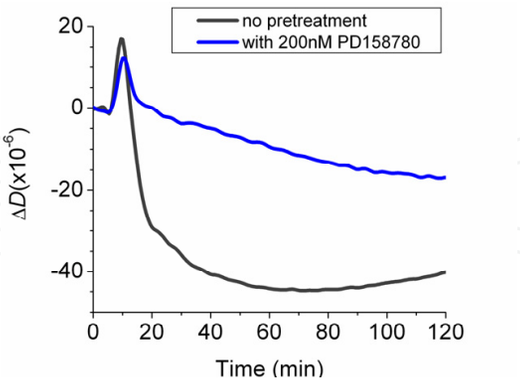

Figure 8. The ΔD response of the cells pretreated with EGFR tyrosine kinase inhibitor, PD158780, showing suppression of 10 nM EGF-induced response. This figure is adapted with permission from (Chen et al., 2011). Copyright 2011, American Chemical Society.

2.3. Remodeling of cytoskeleton probed with fluorescence imaging

The change in mechanical properties of cells has been attributed to remodeling of the cytoskeleton (Kuznetsova et al., 2007), which can be induced by EGF treatment (Rijken et al., 1995; Rijken et al., 1998). Direct evidence of remodeling of the cytoskeleton can be obtained with fluorescence imaging. For this, the cells were first treated with a 10-nM EGF solution for 60 min under the same conditions used for both AFM and QCM-D measurements. The actin filaments of the cytoskeleton were then stained with fluorescently labeled phalloidin and imaged with an inverted fluorescence microscope.

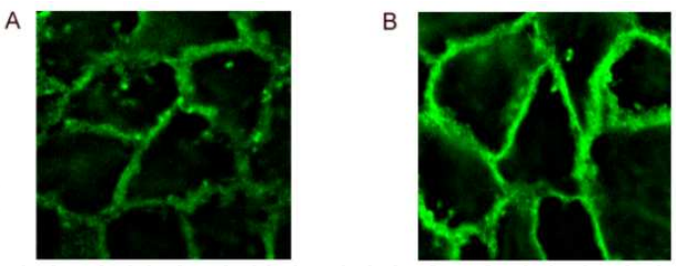

Figure 9. Remodeling of actin filaments in a monolayer of A431 cells induced by the treatment of 10 nM of EGF at 37°C. (A) and (B) show fluorescence-stained actin cortex at the top-half of the cell layer before and after the 60-min treatment with EGF solution, respectively (Yang et al., 2012).

As shown in Figures 9A and B, the top portion of the membrane skeleton exhibited an increase in brightness after the 60-min EGF treatment, indicating an increase of the number and size of the cortical actin filaments. Considering that the cortical actin provides cells with a structural framework, the increase in cortical actin can be assumed responsible for the increased rigidity of the cells, which is manifested as the increase in Young's modulus measured by AFM. In addition, because both energy dissipation and hysteresivity were derived from the same set of force-displacement curves used for determining Young's

modulus of the top region of the cells, it is reasonable to assume that the increases in dissipation and hysteresivity were also related to the increase in cortical actin filaments.

The basal area of the cell monolayer, i.e., the area probed by the QCM-D, was also examined by means of fluorescence imaging. In this case, the stress fibers, which are actin filaments that reside in the bottom portion of the cells and are involved in the formation of focal adhesions attaching the cell to the substrate, were studied. As shown in Figures 10A and 10B, the cells displayed a decrease in amount and size of stress fibers after 60 min of exposure to EGF. Thus the decrease in dissipation observed by means of the QCM-D technique can be reasonably assumed to be related to a reduction in number and size of the actin stress fibers in the basal region of the cells. In addition, this reduction in stress fibers suggests a loss of adhesion and/or contact between the cells and the solid substrate, which is consistent with the EGF-induced cell rounding and retracting that has been reported previously (Chinkers et al., 1979; 1981).

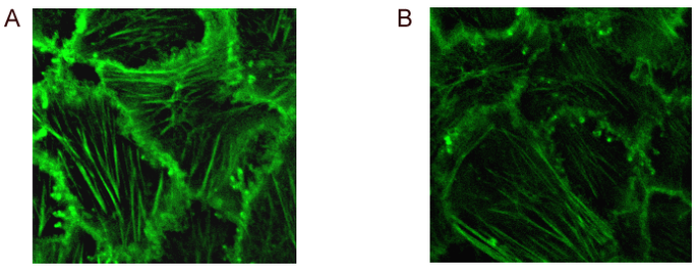

Figure 10. Remodeling of actin filaments in a monolayer of A431 cells induced by the treatment of 10 nM of EGF at 37°C. (A) and (B) show fluorescence-stained stress fibers at the basal area of the cell layer before and after the 60-min EGF treatment, respectively.

2.4. Comparison of AFM with QCM-D results

The two sensitive techniques, AFM and QCM-D, described in this chapter probe two different regions of cells in a monolayer on a solid surface. The nature of the AFM limits it to probing the top surface and immediately underlying volume of individual cells. The nature of the QCM-D limits it to probing the basal areas of the cells in a monolayer. The AFM technique revealed an increase in Young's modulus, energy dissipation, and hysteresivity in response to EGF, while the QCM-D technique revealed a decrease in energy dissipation factor. The fluorescence studies of the changes in actin in the top and bottom surfaces upon exposure to EGF corroborate the differences observed in mechanical properties.

Because both hysteresivity (η) and the change in energy dissipation factor (ΔD) represent the ratio between the dissipated energy and the elastic energy, these two quantities are analogous to each other (Collinsworth et al., 2002). The fact that the opposite trends in them were obtained upon exposure of the cells to EGF is perhaps not unexpected (Figure 5 and 7A). For a monolayer of cells on a solid surface, the bottoms of the cells are restricted in shape by contact with a solid surface, while the tops of the cells have no such restrictions. The bottom surfaces of the cells may therefore exhibit unique responses to the presence of the solid surface and the tops of the cells may not. Thus, contrasts in mechanical properties and in the number, size, and form of actin filaments signify that the mechanical responses of cells to EGFR signaling are regionally specific.

Interestingly, both hysteresivity (η) and the change in energy dissipation factor (ΔD) required about approximately the same amount of time (30 to 40 min) to reach each respective maxima or minimum after the addition of 10 nM of EGF. Such a similarity suggests that both of these regional mechanical responses (ΔD and η) were mediated by closely coupled cell signaling pathway(s) of EGFR.

3. Conclusions

In this chapter, the examples were provided to illustrate how to employ a combination of AFM and QCM-D to characterize the mechanical behavior of cells in response to exposure to EGF. This unique combination allowed the comparative assessment of the upper volume of the cell bodies as well as the basal areas of the cells. Results from both parts of the A431 cells reveal a regionally specific mechanical behavior of the cells, which can be attributed to the distinct cytoskeleton structures utilized by the cells to alter the local structure in response to EGF stimulation. The signaling pathways that mediate the remodeling of the cytoskeleton in the upper volume of the cell bodies are likely closely coupled to those in the basal areas of the cells. There is a clear correlation between the time-dependent mechanical response and the dynamic process of EGFR signaling.

Overall, the combination of AFM and QCM-D is able to provide a more complete and refined mechanical profile of the cells during the dynamic cell signaling process than either technique alone. The use of combined techniques to track real-time cell signaling based on the measurement of cellular mechanical response in a label-free manner is a powerful approach for investigating the role of EGFR in causing abnormal cell behavior. This combined, real-time approach may have the potential to be applied to the study of other types of receptor-mediated cell signaling and trafficking. This approach should contribute to the fundamental understanding of the correlation between cell function and cell mechanical properties.

Author details

Jun Xi, Lynn S. Penn and Jennifer Y. Chen
Drexel University, Department of Chemistry, Philadelphia, PA 19104, USA

Ning Xi and Ruiguo Yang
Michigan State University, Department of Electrical and Computer Engineering, East Lansing, MI 48824, USA

Acknowledgement

This manuscript recapitulates some information from original works that have previously been published in *Analytical Chemistry* (Chen et al., 2011) and *Experimental Cell Research* (Yang et al., 2012). This work was supported in part by NSF Grants IIS-0713346 and DMI-0500372, ONR Grants N00014-04-1-0799 and N00014-07-1-0935, and NIH Grant R43 GM084520. We also appreciate the financial support from Drexel University.

4. References

Addae-Mensah, K. A. & Wikswo, J. P. (2008). Measurement techniques for cellular biomechanics in vitro. *Exp. Biol. Med.,* Vol.233, No.7, pp. 792-809,

Aizawa, H., et al. (2001). Conventional diagnosis of treponema pallidum in serum using latex piezoelectric immunoassay. *Materials Science and Engineering: C,* Vol.17, No.1-2, pp. 127-132, ISSN 0928-4931

Alcaraz, J., Buscemi, L., Grabulosa, M., Trepat, X., Fabry, B., Farr, R. & Navajas, D. (2003). Microrheology of human lung epithelial cells measured by atomic force microscopy. *Biophysical Journal,* Vol.84, No.3, pp. 2071-2079, ISSN 0006-3495

Alessandrini, A., Croce, M. A., Tiozzo, R. & Facci, P. (2006). Monitoring cell-cycle-related viscoelasticity by a quartz crystal microbalance. *Appl. Phys. Lett.,* Vol.88, No.8, pp. 083905/083901-083905/083903, ISSN 0003-6951

Alexopoulos, L. G., Haider, M. A., Vail, T. P. & Guilak, F. (2003). Alterations in the mechanical properties of the human chondrocyte pericellular matrix with osteoarthritis. *Journal of Biomechanical Engineering,* Vol.125, No.3, pp. 323-333,

Bao, G. & Suresh, S. (2003). Cell and molecular mechanics of biological materials. *Nat Mater,* Vol.2, No.11, pp. 715-725, ISSN 1476-1122

Bretscher, A. (1989). Rapid phosphorylation and reorganization of ezrin and spectrin accompany morphological changes induced in a-431 cells by epidermal growth factor. *The Journal of Cell Biology,* Vol.108, No.3, pp. 921-930,

Chen, J. Y., Li, M., Penn, L. S. & Xi, J. (2011). Real-time and label-free detection of cellular response to signaling mediated by distinct subclasses of epidermal growth factor receptors. *Analytical Chemistry,* Vol.83, No.8, pp. 3141-3146, ISSN 0003-2700

Chinkers, M., McKanna, J. A. & Cohen, S. (1979). Rapid induction of morphological changes in human carcinoma cells a-431 by epidermal growth factors. *J. Cell Biol.,* Vol.83, No.1, pp. 260-265,

Chinkers, M., McKanna, J. A. & Cohen, S. (1981). Rapid rounding of human epidermoid carcinoma cells a-431 induced by epidermal growth factor. *J. Cell Biol.,* Vol.88, No.2, pp. 422-429,

Collinsworth, A. M., Zhang, S., Kraus, W. E. & Truskey, G. A. (2002). Apparent elastic modulus and hysteresis of skeletal muscle cells throughout differentiation. *American Journal of Physiology - Cell Physiology,* Vol.283, No.4, pp. C1219-C1227,

Dei Tos, A. P. & Ellis, I. (2005). Assessing epidermal growth factor receptor expression in tumours: What is the value of current test methods. *Eur. J. Cancer,* Vol.41, No.10, pp. 1383-1392, ISSN 0959-8049

Dixon, M. C. (2008). Quartz crystal microbalance with dissipation monitoring: Enabling real-time characterization of biological materials and their interactions. *J. Biomol. Tech.,* Vol.19, No.3, pp. 151-158, ISSN 1943-4731 (Electronic)

Fabry, B., Maksym, G. N., Butler, J. P., Glogauer, M., Navajas, D. & Fredberg, J. J. (2001). Scaling the microrheology of living cells. *Physical Review Letters,* Vol.87, No.14, pp. 148102,

Fletcher, D. A. & Mullins, R. D. (2010). Cell mechanics and the cytoskeleton. *Nature,* Vol.463, No.7280, pp. 485-492, ISSN 0028-0836

Fredberg, J. J. & Stamenovic, D. (1989). On the imperfect elasticity of lung tissue. *Journal of Applied Physiology,* Vol.67, No.6, pp. 2408-2419,

Fredriksson, C., Kihlman, S., Rodahl, M. & Kasemo, B. (1998). The piezoelectric quartz crystal mass and dissipation sensor: A means of studying cell adhesion. *Langmuir,* Vol.14, No.2, pp. 248-251, ISSN 0743-7463

Fung, Y. C. (1984). Structure and stress-strain relationship of soft tissues. *American Zoologist,* Vol.24, No.1, pp. 13-22,

Furtado, L. M. & Thompson, M. (1998). Hybridization of complementary strand and single-base mutated oligonucleotides detected with an on-line acoustic wave sensor. *Analyst,* Vol.123 No.10, pp. 1937 - 1945,

Galbraith, C. G. & Sheetz, M. P. (1997). A micromachined device provides a new bend on fibroblast traction forces. *Proceedings of the National Academy of Sciences of the United States of America,* Vol.94, No.17, pp. 9114-9118,

Galli Marxer, C., Collaud Coen, M., Greber, T., Greber, U. & Schlapbach, L. (2003). Cell spreading on quartz crystal microbalance elicits positive frequency shifts indicative of viscosity changes. *Anal. Bioanal. Chem.,* Vol.377, No.3, pp. 578-586,

Heitmann, V., Reiss, B. & Wegener, J. (2007). The quartz crystal microbalance in cell biology: Basics and applications. *Springer Ser. Chem. Sens. Biosens.,* Vol.5, No.Piezoelectric Sensors, pp. 303-338,

Heitmann, V. & Wegener, J. (2007). Monitoring cell adhesion by piezoresonators: Impact of increasing oscillation amplitudes. *Analytical Chemistry,* Vol.79, No.9, pp. 3392-3400, ISSN 0003-2700

Hoffman, B. D. & Crocker, J. C. (2009). Cell mechanics: Dissecting the physical responses of cells to force. *Annual Review of Biomedical Engineering,* Vol.11, No.1, pp. 259-288,

Hook, F., Rodahl, M., Brzezinski, P. & Kasemo, B. (1998). Energy dissipation kinetics for protein and antibody-antigen adsorption under shear oscillation on a quartz crystal microbalance. *Langmuir,* Vol.14, No.4, pp. 729-734, ISSN 0743-7463

Hook, F., Kasemo, B., Nylander, T., Fant, C., Sott, K. & Elwing, H. (2001). Variations in coupled water, viscoelastic properties, and film thickness of a mefp-1 protein film during adsorption and cross-linking: A quartz crystal microbalance with dissipation monitoring, ellipsometry, and surface plasmon resonance study. *Analytical Chemistry,* Vol.73, No.24, pp. 5796-5804, ISSN 0003-2700

Janmey, P. A. (1998). The cytoskeleton and cell signaling: Component localization and mechanical coupling. *Physiol. Rev.,* Vol.78, No.3, pp. 763-781,

Janmey, P. A., Georges, P. C., Hvidt, S., YuLi, W. & Dennis, E. D. (2007). Basic rheology for biologists, In: *Methods in cell biology,* pp. 1, 3-27, Academic Press, ISBN 0091-679X,

Janshoff, A., Wegener, J., Sieber, M. & Galla, H. J. (1996). Double-mode impedance analysis of epithelial cell monolayers cultured on shear wave resonators. *European Biophysics Journal,* Vol.25, No.2, pp. 93-103,

Janshoff, A., Steinem, C., Sieber, M., el Bayâ, A., Schmidt, M. A. & Galla, H. J. (1997). Quartz crystal microbalance investigation of the interaction of bacterial toxins with ganglioside containing solid supported membranes. *European Biophysics Journal,* Vol.26, No.3, pp. 261-270,

Kasza, K. E., Rowat, A. C., Liu, J., Angelini, T. E., Brangwynne, C. P., Koenderink, G. H. & Weitz, D. A. (2007). The cell as a material. *Curr. Opin. Cell Biol.,* Vol.19, No.1, pp. 101-107, ISSN 0955-0674

Kuznetsova, T. G., Starodubtseva, M. N., Yegorenkov, N. I., Chizhik, S. A. & Zhdanov, R. I. (2007). Atomic force microscopy probing of cell elasticity. *Micron,* Vol.38, No.8, pp. 824-833, ISSN 0968-4328

Le Guillou-Buffello, D., Gindre, M., Johnson, P., Laugier, P. & Migonney, V. (2011). An alternative quantitative acoustical and electrical method for detection of cell adhesion process in real-time. *Biotechnology and Bioengineering,* Vol.108, No.4, pp. 947-962, ISSN 1097-0290

Lee, C.-W., Lin, C.-C., Lin, W.-N., Liang, K.-C., Luo, S.-F., Wu, C.-B., Wang, S.-W. & Yang, C.-M. (2007). Tnf-{alpha} induces mmp-9 expression via activation of src/egfr, pdgfr/pi3k/akt cascade and promotion of nf-{kappa}b/p300 binding in human tracheal smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol,* Vol.292, No.3, pp. L799-812,

Lee, H., Contarino, M., Umashankara, M., Schön, A., Freire, E., Smith, A., Chaiken, I. & Penn, L. (2010). Use of the quartz crystal microbalance to monitor ligand-induced conformational rearrangements in hiv-1 envelope protein gp120. *Anal. Bioanal. Chem.,* Vol.396, No.3, pp. 1143-1152, ISSN 1618-2642

Li, F., Wang, J. H. C. & Wang, Q.-M. (2008). Thickness shear mode acoustic wave sensors for characterizing the viscoelastic properties of cell monolayer. *Sensors and Actuators B: Chemical,* Vol.128, No.2, pp. 399-406, ISSN 0925-4005

Marx, K. A., Zhou, T., Montrone, A., McIntosh, D. & Braunhut, S. J. (2005). Quartz crystal microbalance biosensor study of endothelial cells and their extracellular matrix following cell removal: Evidence for transient cellular stress and viscoelastic changes during detachment and the elastic behavior of the pure matrix. *Anal. Biochem.,* Vol.343, No.1, pp. 23-34, ISSN 0003-2697

Matsuda, T., Kishida, A., Ebato, H. & Okahata, Y. (1992). Novel instrumentation monitoring in situ platelet adhesivity with a quartz crystal microbalance. *ASAIO Journal,* Vol.38, No.3, pp. M171-M173, ISSN 1058-2916

McManus, M. J., Boerner, J. L., Danielsen, A. J., Wang, Z., Matsumura, F. & Maihle, N. J. (2000). An oncogenic epidermal growth factor receptor signals via a p21-activated kinase-caldesmon-myosin phosphotyrosine complex. *Journal of Biological Chemistry,* Vol.275, No.45, pp. 35328-35334,

Melzak, K. A., Moreno-Flores, S., Lopez, A. E. & Toca-Herrera, J. L. (2011). Why size and speed matter: Frequency dependence and the mechanical properties of biomolecules. *Soft Matter,* Vol.7, No.2, pp. 332-342, ISSN 1744-683X

Nimeri, G., Fredriksson, C., Elwing, H., Liu, L., Rodahl, M. & Kasemo, B. (1998). Neutrophil interaction with protein-coated surfaces studied by an extended quartz crystal microbalance technique. *Colloids and Surfaces B: Biointerfaces,* Vol.11, No.5, pp. 255-264, ISSN 0927-7765

Ono, M. & Kuwano, M. (2006). Molecular mechanisms of epidermal growth factor receptor (egfr) activation and response to gefitinib and other egfr-targeting drugs. *Clinical Cancer Research,* Vol.12, No.24, pp. 7242-7251,

Otto, K., Elwing, H. & Hermansson, M. (1999). Effect of ionic strength on initial interactions of escherichia coli with surfaces, studied on-line by a novel quartz crystal microbalance technique. *J. Bacteriol.,* Vol.181, No.17, pp. 5210-5218,

Papakonstanti, E. A. & Stournaras, C. (2008). Cell responses regulated by early reorganization of actin cytoskeleton. *FEBS Lett.,* Vol.582, No.14, pp. 2120-2127, ISSN 0014-5793

Pax, M., Rieger, J., Eibl, R. H., Thielemann, C. & Johannsmann, D. (2005). Measurements of fast fluctuations of viscoelastic properties with the quartz crystal microbalance. *The Analyst,* Vol.130, No.11, pp. 1474-1477,

Radmacher, M. (1997). Measuring the elastic properties of biological samples with the afm. *IEEE Engineering in Medicine and Biology Magazine,* Vol.16, No.2, pp. 47,

Radmacher, M. (2007). Studying the mechanics of cellular processes by atomic force microscopy, In: *Methods in cell biology*, YuLi W. & Dennis E. D., pp. 347-372, Academic Press, ISBN 0091-679X,

Redepenning, J., Schlesinger, T. K., Mechalke, E. J., Puleo, D. A. & Bizios, R. (1993). Osteoblast attachment monitored with a quartz crystal microbalance. *Analytical Chemistry,* Vol.65, No.23, pp. 3378-3381, ISSN 0003-2700

Reipa, V., Almeida, J. & Cole, K. D. (2006). Long-term monitoring of biofilm growth and disinfection using a quartz crystal microbalance and reflectance measurements. *Journal of Microbiological Methods,* Vol.66, No.3, pp. 449-459, ISSN 0167-7012

Rewcastle, G. W., Palmer, B. D., Thompson, A. M., Bridges, A. J., Cody, D. R., Zhou, H., Fry, D. W., McMichael, A. & Denny, W. A. (1996). Tyrosine kinase inhibitors. 10. Isomeric 4-[(3-bromophenyl)amino]pyrido[d]- pyrimidines are potent atp binding site inhibitors of the tyrosine kinase function of the epidermal growth factor receptor. *J. Med. Chem.*, Vol.39, No.9, pp. 1823-1835, ISSN 0022-2623

Ridley, A. J. (1994). Membrane ruffling and signal transduction. *BioEssays,* Vol.16, No.5, pp. 321-327, ISSN 1521-1878

Rijken, P. J., Hage, W. J., van Bergen en Henegouwen, P. M., Verkleij, A. J. & Boonstra, J. (1991). Epidermal growth factor induces rapid reorganization of the actin microfilament system in human a431 cells. *J. Cell Sci.,* Vol.100, No.3, pp. 491-499,

Rijken, P. J., Post, S. M., Hage, W. J., van Bergen en Henegouwen, P. M. P., Verkleij, A. J. & Boonstra, J. (1995). Actin polymerization localizes to the activated epidermal growth factor receptor in the plasma membrane, independent of the cytosolic free calcium transient. *Experimental Cell Research,* Vol.218, No.1, pp. 223-232, ISSN 0014-4827

Rijken, P. J., van Hal, G. J., van der Heyden, M. A. G., Verkleij, A. J. & Boonstra, J. (1998). Actin polymerization is required for negative feedback regulation of epidermal growth factor-induced signal transduction. *Experimental Cell Research,* Vol.243, No.2, pp. 254-262, ISSN 0014-4827

Rodahl, M., Hook, F. & Kasemo, B. (1996). Qcm operation in liquids: An explanation of measured variations in frequency and q factor with liquid conductivity. *Analytical Chemistry,* Vol.68, No.13, pp. 2219-2227, ISSN 0003-2700

Rodahl, M. & Kasemo, B. (1996). A simple setup to simultaneously measure the resonant frequency and the absolute dissipation factor of a quartz crystal microbalance. *Rev. Sci. Instrum.,* Vol.67, No.9, pp. 3238-3241, ISSN 0034-6748

Rodahl, M., Hook, F., Fredriksson, C., Keller, C. A., Krozer, A., Brzezinski, P., Voinova, M. & Kasemo, B. (1997). Simultaneous frequency and dissipation factor qcm measurements of biomolecular adsorption and cell adhesion. *Faraday Discuss*, No.107, pp. 229-246, ISSN 1359-6640 (Print)

Satcher Jr, R. L. & Dewey Jr, C. F. (1996). Theoretical estimates of mechanical properties of the endothelial cell cytoskeleton. *Biophysical Journal,* Vol.71, No.1, pp. 109-118, ISSN 0006-3495

Sauerbrey, G. (1959). Verwendung von schwingquarzen zur wägung dünner schichten und zur mikrowägung. *Zeitschrift für Physik A Hadrons and Nuclei,* Vol.155, No.2, pp. 206-222,

Scaltriti, M. & Baselga, J. (2006). The epidermal growth factor receptor pathway: A model for targeted therapy. *Clinical Cancer Research,* Vol.12, No.18, pp. 5268-5272,

Schillers, H., Wälte, M., Urbanova, K. & Oberleithner, H. (2010). Real-time monitoring of cell elasticity reveals oscillating myosin activity. *Biophysical Journal,* Vol.99, No.11, pp. 3639-3646, ISSN 0006-3495

Schliwa, M. (1982). Action of cytochalasin d on cytoskeletal networks. *J. Cell Biol.,* Vol.92, No.1, pp. 79-91,

Silva, C. M. (2004). Role of stats as downstream signal transducers in src family kinase-mediated tumorigenesis. *Oncogene,* Vol.23, No.48, pp. 8017-8023, ISSN 0950-9232

Smith, B. A., Tolloczko, B., Martin, J. G. & Grutter, P. (2005). Probing the viscoelastic behavior of cultured airway smooth muscle cells with atomic force microscopy: Stiffening induced by contractile agonist. *Biophysical Journal,* Vol.88, No.4, pp. 2994-3007, ISSN 0006-3495

Sollich, P. (1998). Rheological constitutive equation for a model of soft glassy materials. *Physical Review E,* Vol.58, No.1, pp. 738,

Song, W., Wu, J., Ge, G. & Lin, Q. (2008). Two domains of the epidermal growth factor receptor are involved in cytoskeletal interactions. *Biochemical and Biophysical Research Communications,* Vol.370, No.4, pp. 589-593, ISSN 0006-291X

Stamenovic, D. (2005). Effects of cytoskeletal prestress on cell rheological behavior. *Acta Biomaterialia,* Vol.1, No.3, pp. 255-262, ISSN 1742-7061

Svoboda, K. & Block, S. M. (1994). Biological applications of optical forces. *Annual Review of Biophysics and Biomolecular Structure,* Vol.23, No.1, pp. 247-285,

Touhami, A., Nysten, B. & Dufrene, Y. F. (2003). Nanoscale mapping of the elasticity of microbial cells by atomic force microscopy. *Langmuir,* Vol.19, No.11, pp. 4539-4543, ISSN 0743-7463

Usami, S., Chen, H.-H., Zhao, Y., Chien, S. & Skalak, R. (1993). Design and construction of a linear shear stress flow chamber. *Annals of Biomedical Engineering,* Vol.21, No.1, pp. 77-83,

Voinova, M. V., Jonson, M. & Kasemo, B. (2004). On dissipation of quartz crystal microbalance as a mechanical spectroscopy tool. *Spectroscopy,* Vol.18, No.4, pp. 537-544,

Wang, N., Butler, J. P. & Ingber, D. E. (1993). Mechanotransduction across the cell surface and through the cytoskeleton. *Science,* Vol.260, No.5111, pp. 1124-1127,

Wegener, J., Janshoff, A. & Galla, H. J. (1998). Cell adhesion monitoring using a quartz crystal microbalance: Comparative analysis of different mammalian cell lines. *European Biophysics Journal,* Vol.28, No.1, pp. 26-37,

Wirtz, D. (2009). Particle-tracking microrheology of living cells: Principles and applications. *Annual Review of Biophysics,* Vol.38, No.1, pp. 301-326,

Yang, R., Chen, J. Y., Xi, N., Lai, K. W. C., Qu, C., Fung, C. K. M., Penn, L. S. & Xi, J. (2012). Characterization of mechanical behavior of an epithelial monolayer in response to epidermal growth factor stimulation. *Experimental Cell Research,* Vol.318, No.5, pp. 521-526, ISSN 0014-4827

Yasmeen, A., Bismar, T. A. & Al Moustafa, A.-E. (2006). Erbb receptors and epithelial-cadherin-catenin complex in human carcinomas. *Future Oncology,* Vol.2, No.6, pp. 765-781,