Specific Mechanical and Structural Responses of Cortical and Cytosolic Cytoskeleton in Living Adherent Cells*

Valérie M. LAURENT**, Redouane FODIL***,
Patrick CAÑADAS***, Emmanuelle PLANUS***
and Daniel ISABEY***

We studied the relation between actin structural changes and cytoskeleton mechanical properties in living adherent epithelial alveolar cells, before and during actin depolymerization. The mechanical response of adherent alveolar epithelial cells was measured using magnetic twisting cytometry and a two-component model representing the cortical and cytosolic elastic components at equilibrium. Chemiluminescent staining of the actin cytoskeleton was performed in the same living cells to estimate the intracellular actin density distribution for each cytoskeleton component. We found that (i) cytoskeleton alterations induced by actin depolymerization differed between the cortical and cytosolic cytoskeleton components (e.g., -30% and -49% , respectively, at a stress of 31 Pa) and that (ii) the concomitant change in actin distribution was also different (e.g., actin volume decrease was -7% and -19% for the cortical and cytosolic components, respectively).

Key Words: Magnetic Twisting Cytometry, Actin Depolymerization, Staining of Actin, GFP

1. Introduction

The cytoskeleton (CSK) plays an important role in stabilizing cell shape under a variety of environmental and intracellular conditions^{(1)–(3)}. Its involvement in many cell functions such as spreading, crawling, and cytokinesis has been well demonstrated^{(4)–(7)}. The CSK is a complex, heterogeneous molecular lattice of biopolymers⁽⁸⁾. Magnetic twisting cytometry (MTC) is one of the few micromanipulation methods

that specifically measures the CSK response⁽⁹⁾. The three basic types of interconnected CSK filaments (actin filaments or F-actin, microtubules, and intermediate filaments), which are the major components of the cytoplasm, have been shown to be responsible for the “solid” response and “prestressed” behavior of the CSK^{(10)–(13)}. We have reported a marked decrease in CSK stiffness upon exposure to an F-actin depolymerizing agent. This decrease was probably related to a drop in the internal tension⁽¹³⁾ produced by tensed actin filaments^{(10),(14)–(16)}. In an MTC study of CSK mechanical properties, we recently found that the CSK mechanical response could be partitioned in two components⁽¹⁷⁾, namely, a “cytosolic” CSK component made of biopolymers that link and transmit forces through large regions of the cytoplasm, such as from surface adhesion receptors to the nucleus⁽¹⁸⁾; and a “cortical” CSK component that acts at a smaller scale to play a major role in cell motility, endocytosis, and local accommodation of the cell to stress^{(19)–(21)}. The “cortical” cytoskeleton, constituted by the

* Received 16th July, 2002 (No. 02-5100)

** Cellular Biophysics and Biomechanics Laboratory, Swiss Federal Institute of Technology, Lausanne, Switzerland/Fonctions Cellulaires et Moléculaires de l'Appareil Respiratoire et des Vaisseaux, Inserm UMR S 492, Facultés de Médecine et des Sciences et Technologie Paris XII, Créteil, France. E-mail: valerie.laurent@epfl.ch

*** Fonctions Cellulaires et Moléculaires de l'Appareil Respiratoire et des Vaisseaux, Inserm UMR S 492, Facultés de Médecine et des Sciences et Technologie Paris XII, Créteil, France

spectrin/ankyrin/actin network, is coupled with the inner face of the cell membrane^{(19),(22),(23)}. Surprisingly, it has received much less research attention in adherent cells than in non adherent cells although it has been implicated in many cellular functions for both adherent and non adherent cells^{(24)–(26)}. A reasonable hypothesis is that the cortical and cytosolic CSK components differ in their response to actin depolymerizing drugs. To test this hypothesis, direct experimental evidence that the two components exhibit different responses must be obtained, and this must be done in living cells. Previous studies of the effects of actin depolymerization on actin structure used cells fixed by fluorescent phallotoxin^{(13),(27)}.

The aim of the present study was to look for a relation between structural changes and mechanical properties of living adherent cells before and during treatment with the actin depolymerizing agent cytochalasin D. The mechanical response of adherent alveolar epithelial cells was measured using MTC. The corresponding elastic contribution was then extracted from the viscoelastic response using a two-component model representing the cortical and cytosolic components. To investigate the corresponding changes in actin density distribution while keeping the cultured cells alive, we used a pEGFP-Actin fusion protein transfected in cultured epithelial cells to achieve chemiluminescent staining of the actin CSK. Then, we compared the actin density distribution, assessed by cumulated and planar views near the basal face of the cells, to changes in mechanical properties before and after actin depolymerization.

2. Materials and Methods

2.1 A549 alveolar epithelial cell culture

A549 human alveolar epithelial cells (American Type Culture Collection, Rockville, MD) were grown to confluence in DMEM containing 10% FBS, 2 mM L-glutamine, 50 IU/ml penicillin, and 50 µg/ml streptomycin, and incubated in a 5% CO₂ – 95% air atmosphere. Routine subcultures (passages 89 to 92) were performed at 1/20 split ratios by incubation with 0.025 g/100 ml trypsin–0.02 g/100 ml EDTA in calcium- and magnesium-free PBS for 10 min at 37°C. For MTC experiments, 96-well bacteriologic dishes were coated with type I collagen at a concentration of 5 µg/cm² for 3 hours at room temperature. Cells were plated at a density of 50 000 per well in complete medium with serum for 24 hours and then incubated in serum-free medium with 1% BSA for 30 minutes.

2.2 Staining and observation of actin in living cells

Staining of actin in living A549 human epithelial cells was performed with the green fluorescent protein

(GFP) pEGFP-Actin fusion protein. Transient plasmid transfection into A549 cells was achieved by electroporation. Plasmids (~32 µg) encoding for pEGFP-βactin (Clontech[®]) were transfected into 6.10⁶ cells using an electroporation system (Easyject Plus[®] EquiBio, Kent, UK). Fluorescence produced by pEGFP-βactin plasmid expression was detected 48 hours after transfection.

Cells transfected with pEGFP-βactin were cultured in complete medium on round glass coverslips previously coated with type I collagen and incubated in serum-free medium with 1% BSA for 30 minutes before observation under an LSM 410 inverted confocal microscope (Zeiss, Rueil-Malmaison, France) that had two internal helium-neon lasers and one external argon ion laser. GFP was excited by a blue 500 nm-laser source and observed through a 100×/1.3 numeric aperture Plan Neofluor objective. Optical layers 1-µm thick with a spatial resolution of 400 – 500 µm were used. Image processing was performed using LSM 410 software.

2.3 3D-reconstruction of actin structure

To remove the distortion due to the optical system of the confocal microscope, a 3D deconvolution process was performed on the stack of gray-level images (8 bits) by the software module AxioVision 3.0.6 (Carl Zeiss, NY USA). The deconvolution process uses a mathematical algorithm that takes into account the theoretical Point Spread Function calculated for each acquisition parameter. The fixed image data are implemented at the right scale by Amira 2.3 software (TGS Inc., CA USA), which uses the threshold segmentation method to extract cell contours. The contours of external and internal subcellular structures corresponding to two different levels of fluorescence intensity were defined using the curve of the “logarithmic” decrease in cumulated pixels of the stack images versus the gray level (0 to 255). The 3D-reconstruction was performed by the Generalized Marching Cube algorithm of the Amira software, which uses a triangulation method to generate an isosurface from segmented optical planes. Calculation of the volume and visualization of each CSK-actin component were performed using 3D-Studio Max v3.1 software (Kinetix, CA USA).

2.4 Mechanical measurements by magnetic twisting cytometry

Initially proposed by Wang et al.⁽⁹⁾, MTC applies a defined mechanical stress to adherent cell cytoskeleton via ferromagnetic beads linked to transmembrane receptors. The MTC device used for the present study was manufactured at our laboratory, as previously described^{(13),(14)}. Carboxyl ferromagnetic beads (4.0 – 4.5 µm diameter, Spherotec Inc., IL USA)

were coated with arginine-glycin-aspartic acid (RGD) peptide, as recommended by the manufacturer (Telios Pharmaceuticals Inc., CA USA). Before use, the coated beads were incubated in serum-free medium supplemented with 1% BSA for at least 30 minutes at 37°C, to block nonspecific binding. The beads were then added to the cells (40 µg per well) for 20 minutes at 37°C in a 5% CO₂ + 95% air incubator. Unbound beads were washed away with serum-free medium containing 1% BSA and 25 mM HEPES. The microbeads were then magnetized using a 0.15-T uniform magnetic pulse (150 µs). This magnetic field was parallel to the monolayer of adherent cells. A magnetic torque was then created by Helmholtz coils, which generate a uniform vertical magnetic field (B_{tw}) during 60 seconds ($B_{tw} \leq 6.3$ mT, i.e., much weaker than the magnetizing field). The bead rotation induced by the magnetic torque reflected the CSK response to mechanical stresses. To determine the magnitude of bead rotation (θ), a magnetometer (Magnetoscop 1.068, Förster France, Gentilly, France) was used to continuously detect the component of the remanent magnetic field in the horizontal plane $B(t)$ (≤ 2 nT). It was assumed that the average bead deviation could be approximated by the arc cosine of the projected magnetic field averaged over all the beads ($\sim 200\,000$). Typically, over 1 minute, θ increases sharply then continues to rise more slowly toward a plateau indicating stability. Thus, stiffness can be measured at the end of 1 minute of magnetic field application. The time-course of this angular deviation was analyzed using a two-component rheological model (a series of two Voigt models) replicating the cortical and cytosolic CSK responses. Curve fitting was recently found to be much better with this two-component model than with the previously used single component model⁽¹⁷⁾. Because θ nearly always reaches a plateau during MTC measurements^{(6),(9),(13)–(15)}, a “solid” model (such as the double Voigt model) fits the CSK response much better than does a “fluid” model. This was also true with a refined “fluid” model such as a Kelvin body in series with a dashpot element already used to fit data obtained with a magnetic bead microrheology method, which yet greatly differs from MTC in terms of the force applied⁽²⁸⁾.

2.5 Effect of actin depolymerization: cytochalasin D treatment

To study the effect of actin depolymerization on the mechanical properties of the cortical and cytosolic actin-CSK components, we used low concentrations of cytochalasin D, a compound that specifically depolymerizes F-actin CSK. This drug has been previously reported to decrease actin-CSK internal tension

without markedly changing cell attachment⁽¹³⁾. The two-component model was used to analyze the CSK response in untreated epithelial cells and in cells treated by cytochalasin D. Cells were plated either in 96-well plates or on glass coverslips pre-coated with type 1 collagen. Adherent cells were treated 24 h later with a low concentration of cytochalasin D (1 µg/ml) before MTC measurements or confocal microscope examination.

3. Results

3.1 Mechanical results

The mechanical properties of each CSK component of living alveolar epithelial cells are reported in Fig. 1 A – C for different levels of applied stress (i.e., from 13 to 38 Pa, which are values easily obtainable by changing the current intensity in the Helmholtz coils of the perpendicular field). Increasing the applied stress resulted in an increase in stiffness of both CSK components (except for the cytosolic component at 38 Pa, but this was ascribable to a measurement artifact). The increase was more marked for the cytosolic component below 38 Pa than for the cortical component. For all tested stress levels, stiffness of the cytosolic CSK component (Fig. 1A) was about twice as high as that of the cortical component (Fig. 1B). Actin filament depolymerization by cytochalasin D produced significant alterations, which differed between the two CSK components: for instance, with 31 Pa of stress, stiffness decreased from 83 Pa to 58 Pa (–30%) for the cortical component and from 204 Pa to 104 Pa (–49%) for the cytosolic component. To quantify the cytochalasin D-induced alteration in the actin filament network (which was greatest after 6 minutes of treatment), we plotted the differences between the stiffness values obtained before and during cytochalasin D treatment (Fig. 1C). At all tested stress levels (except perhaps the lowest), the contribution of actin filament was significantly greater for the cytosolic than for the cortical component. Moreover, the stress-dependence of actin filament contribution differed between the two components, suggesting major structural differences.

3.2 Structural results

The structural alterations in the cortical and cytosolic actin networks induced by cytochalasin D were studied directly in the same living alveolar epithelial cells by examining fluorescent images in which F and G actin were revealed by the expression of pEGFP-Actin fusion protein. First, F and G actin were stained with pEGFP-Actin fusion protein. Cumulative confocal microscopy images of fluorescence intensity, i.e., F and G actin density, in living A549 human epithelial cells are shown in Fig. 2. We

Fig. 1 Stiffness (in Pascals) of living adherent epithelial alveolar cells, for four levels of applied stress: $\sigma_0=13, 23, 31$ and 38 Pa. The elastic CSK response measured by magnetic twisting cytometry is partitioned into a cortical and a cytosolic elastic component. Values are mean $\pm$ SD and $n=8$ wells for each point. “*” indicates a significant difference with the Student T-test (i.e., $P<0.05$, except in C where the limit of significance used is $P<0.06$). (A): stiffness of the cortical CSK component before (closed bars) and during (open bars) cytochalasin D treatment ($*P<0.05$). (B): stiffness of the cytosolic CSK component before (closed bars) and during cytochalasin D treatment (open bars) ($*P<0.05$). (C): the contribution of actin to stiffness for the cortical CSK component (dark gray) and the cytosolic CSK component (light gray) is the difference between CSK stiffness values (ΔE in Pa) measured before (closed bars in A and B) and during cytochalasin D treatment (open bars in A and B). Bar errors are increased because of addition of the errors on each term of the difference ($*P<0.06$).

compared findings before (in Fig. 2A) and during (in Fig. 2B) cytochalasin D treatment. We also studied actin distribution before and during cytochalasin D treatment in a selected horizontal plane located near the basal face of the cell, namely, the plane where the cell adhered to the fibronectin-coated glass (Fig. 3 A and 3B). The planar contours of the cortical and cytosolic CSK actin networks were visualized using two different levels of fluorescence intensity, i.e., attributing to the cortical actin CSK the lowest detectable fluorescence intensity (in gray) and to the cytosolic actin CSK an arbitrary intermediate level of higher fluorescence intensity (in black). The idea behind is to consider that actin density is somehow related to the degree of actin assembly. Compared to

the pretreatment actin distribution (Fig. 3A), major changes occurred for both the cortical and cytosolic CSK component during cytochalasin D treatment (Fig. 3B). Cytosolic actin distribution was deeply affected by cytochalasin D treatment, whereas cortical actin distribution showed only minor changes, except in the periphery. Spatial and structural changes in actin distribution are shown in Fig. 3C and 3D, which display the difference in the planar distributions of actin before and during cytochalasin D treatment for the cortical (Fig. 3C) and cytosolic (Fig. 3D) CSK components. As expected, the main effect of actin depolymerization was a decrease in actin assembly. The filopodia extensions clearly visible in the cortical CSK component before treatment (Fig. 3A)

Fig. 2 Cumulative confocal microscopy view of actin density in living A549 human epithelial cells before (in A) and during (in B) cytochalasin D treatment. Staining of F and G actin was performed with a pEGFP-Actin fusion protein.

Fig. 3 Staining of F and G actin with a pEGFP-Actin fusion protein is presented in an optical plane located near the basal face of the cell (i.e., less than $1\ \mu\text{m}$ from the substrate) before (in A) and during (in B) cytochalasin D treatment. The spatial distribution of actin is shown for the lower actin density representing the cortical CSK (in gray) and for a higher density level representing the cytosolic CSK (in black). Changes in actin distribution for the cortical (in C) and cytosolic (in D) components are obtained by subtracting the planar distribution shown in B from that shown in A, for the cortical and cytosolic CSK components separately. This difference represents the actin balance before and during actin depolymerization in the basal cellular plane.

disappeared almost completely during cytochalasin D treatment ("actin" in Fig. 3C). Moreover, the disappearance of actin at the periphery of the cell (Fig. 3C) suggests slight cellular retraction with no change in overall cell shape. Re-concentration of actin in the cortical CSK did not occur, except in a few spots (Fig. 3C). In contrast, the actin in the cytosolic CSK underwent considerable rearrangement. Actin disappeared almost everywhere in the cytosolic component. Thus, the highly polymerized cytosolic component was more deeply altered by actin depolymerization than was the cortical component.

These results obtained in the basal plane were confirmed for the entire cell by measuring actin volume changes in the cortical and cytosolic CSK on 3-D reconstructions before and during cytochalasin D treatment (see Methods). The overall actin volume decreased by 7% in the cortical component and 19% in the cytosolic component during cytochalasin D treatment.

4. Discussion

The fundamental role played by the internal cytoskeleton (i.e., the interconnected biopolymers that constitute the actin filament assemblies, microtubules, and intermediate filaments) in the mechanical behavior of living cells has been previously established on the basis of (i) theoretical arguments brought by elastic and viscoelastic tensegrity models whose behavior resembles cell responses^{(10),(12),(13),(16),(29),(30)} and (ii) experimental evidence that stiffness of cells twisted by RGD-coated beads decreases markedly after actin filament depolymerization^{(9),(31)}. In the present study, we found that the (quasi-static) elastic component of the CSK response in living epithelial cells differed between the cortical and cytosolic components regarding sensitivity to actin filament depolymerization: the more polymerized cytosolic component was more sensitive to cytochalasin D treatment than was the cortical component. This result strongly suggests differences in the contribution of actin filament to the stiffness of the cortical and cytosolic CSK components and supports the differences in actin structural organization reported in a previous study⁽³²⁾. The cortical CSK component comprises a large number of short elements, and the cytosolic component a smaller number of longer and larger elements. Reconstruction of the cortical actin structure from stereo images showed that actin filaments were about 90 nm thick and 100 nm long on average in endothelial cells⁽²³⁾. In addition, from the density of cortical actin filaments per unit area reported by Cheng et al., we estimated that the number of actin filaments in the cortical component

was between 10^4 and 10^5 . By contrast, stress fiber length in the cytosolic component has been estimated at about $90 \pm 40 \mu\text{m}$ by Katoh et al.⁽³³⁾, with some fibers having estimated lengths of only $10 \mu\text{m}$, and the number of actin filament assemblies at 50 to 500. Both tensegrity model data and experimental data suggest that tension may differ markedly between the cortical and cytosolic components⁽³²⁾. The present study of changes in the spatial distribution of actin induced by actin depolymerization provides independent confirmation of these behavioral, or structural, differences between the cortical and cytosolic CSK components. To our knowledge, direct imaging of actin remodeling induced by cytochalasin D treatment in living cells has not been reported previously. We quantitated the structural alterations in each CSK component by estimating the volume of each actin CSK substructure. Our results confirm that the cortical and cytosolic CSK components differ in their actin content (Fig. 1C) and, therefore, in their prestress level, hence justifying the two-component CSK model. Interestingly, the cortical remodeling induced by actin depolymerization was limited to slight retraction with no change in cell shape, whereas corresponding cytosolic remodeling occurred throughout the CSK structure. This heterogeneity in the response of actin substructures to a depolymerizing agent is not surprising. In a recent study, Katoh et al.⁽³⁴⁾ found that stress control at the central and peripheral levels were regulated by different mediators, namely, Rho-kinase for the central stress fibers and myosin light-chain kinase (MLCK) for the peripheral stress fibers. Given that the difference between central and peripheral stress fibers is the size (and/or number) of filaments involved in actin filament assembly while the ultrastructure remains the same⁽³⁴⁾, it is not inconsistent to find in the present study that a given depolymerizing agent such as cytochalasin D has different effects on two actin components, i.e., the cortical and the cytosolic, which differ in terms of F-actin assembly.

One of the limitations of the present comparison of structural features and mechanical properties is that actin structure was examined near the basal face of the cells, i.e., near the points of adhesion of the CSK to the extracellular matrix, whereas the mechanical analysis refers mainly to beads located mainly on the top of the cells. It should be pointed out, however, that (i) the coated bead acts locally as a small extracellular matrix (ECM), hence the bead can sense the proximal — also submembranous — cortical CSK component whatever the bead location in the cell and (ii) the physical connection between the cortical and cytosolic CSK and the bead or substrate occurs at focal adhesion plaques forming molecular junctions,

hence the bead can sense mechanical properties of a cytosolic structure that is in series with the cortical structure. In other words, the properties of the cortical CSK component at the site used in our study probably reflect the properties of the basal cortical CSK. Figure 1A and 1B shows that both CSK components exist near the basal face and that depolymerization alters the cytosolic CSK and, to a lesser extent, the cortical CSK structure.

Another limitation of our study is that we analyzed only actin, disregarding the other CSK filaments that contribute to the overall cell mechanical response. Among the three CSK biopolymers characterized by specific ultrastructural features, mechanical properties, and molecular interactions with extracellular ligands, actin filaments and crosslinking proteins, the filaments appear to make a major contribution to the mechanical properties of the cytoplasm^{(3),(5),(11),(12),(35)}. Therefore, staining only F- and G-actin, not other filament types, provides a satisfactory map of the intracellular density of the main structural elements. Note that estimating the volume of actin from 3D-reconstructions for two actin density levels, i.e., for the cortical and the cytosolic CSK, directly confirmed earlier mechanical data on the effect of actin depolymerization in living cells^{(13),(15)}.

5. Conclusion

The two major contributions of the present study are: (i) the introduction, in the classical analysis of the mechanical CSK response by a "solid" elastic (or viscoelastic) model⁽⁹⁾, of a concept of heterogeneous mechanical behavior, with two elastic CSK components instead of one, (ii) the direct validation of this concept by images showing structural heterogeneity for two levels of actin density. Such a two-component CSK model has been suggested earlier, but not quantified. For instance, in a recent article, Ingber⁽³⁶⁾ showed that the internal, tensed microfilament-microtubule-intermediate filament cytoskeleton characterized by tensile and compressive elements was surrounded by an elastic submembranous cytoskeleton (e.g., actin-ankyrin-spectrin network) and its associated lipid bilayer. This submembranous cytoskeleton component is also stabilized to a certain extent by prestress whose data presently reported in Fig. 1C provide a first estimate (the method used resembles to that previously used by Planus et al.⁽¹⁴⁾ and Pourati et al.⁽¹⁵⁾). Prestress in the cortical CSK may be generated actively through the cell contractile apparatus or passively through osmotic forces acting across the cell membrane or, on a smaller scale, by traction forces created by filament polymerization. Cortical tension may be responsible for stiffening of

the plasma membrane^{(37),(38)} and for many plasma membrane-associated cell functions such as exocytosis^{(39),(40)} and regulation of stretch-induced calcium signals⁽⁴¹⁾. It should be underlined that the smaller rigidity values measured in the cortical component are consistent with the marked adaptability and responsiveness to environmental changes required for the cortical CSK function. By contrast, the higher rigidity values measured in the cytosolic CSK appear consistent with the higher level of filament cross-linking in the cytoplasm and also with the cellular distribution of actin bundles, or "stress fibers", which are thought to protect the cell against external mechanical stresses^{(5),(42)}.

Acknowledgements

The authors gratefully acknowledge Matthieu Rambaudon for his technical assistance for magnetic twisting cytometry measurements. Redouane Fodil and Emmanuelle Planus received a grant (ACI Jeunes Chercheurs 2001 #2094) from the French Ministry of Research. This study was supported by the Institut National de la Santé et de la Recherche Médicale.

References

- (1) Huang, S. and Ingber, D.E., A Discrete Cell Cycle Checkpoint in Late G(1) that is cytoskeleton-dependent and MAP kinase (Erk)-independent, *Exp Cell Research*, Vol. 275, No. 2 (2002) pp. 255-264.
- (2) Shafrir, Y. and Forgacs, G., Mechanotransduction through the Cytoskeleton, *Am. J. Physiol. Cell Physiol.*, Vol. 282, No. 3 (2002), pp. C479-486.
- (3) Ingber, D.E., Prusty, D., Sun, Z., Betensky, H. and Wang, N., Cell Shape, Cytoskeletal Mechanics, and Cell Cycle Control in Angiogenesis, *J. Biomech.*, Vol. 28, No. 12 (1995), pp. 1471-1484.
- (4) Stossel, T.P., On the Crawling of Animal Cells, *Science*, Vol. 260, No. 5111 (1993), pp. 1086-1094.
- (5) Wang, N. and Ingber, D.E., Control of Cytoskeletal Mechanics by Extracellular Matrix, Cell Shape, and Mechanical Tension, *Biophys. J.*, Vol. 66, No. 6 (1994), pp. 2181-2189.
- (6) Schmidt, C.E., Dai, J., Lauffenburger, D.A., Sheetz, M.P. and Horwitz, A.F., Integrin-Cytoskeletal Interactions in Neuronal Growth Cones, *J. Neurosci.*, Vol. 15, No. 5, Pt 1 (1995), pp. 3400-3407.
- (7) Heidemann, S.R. and Buxbaum, R.E., Mechanical Tension as a Regulator of Axonal Development, *Neurotoxicology*, Vol. 15, No. 1 (1994), pp. 95-107.
- (8) Janmey, P.A., The Cytoskeleton and Cell Signaling: Component Localization and Mechanical Coupling, *Physiol. Rev.*, Vol. 78, No. 3 (1998), pp. 763-781.
- (9) Wang, N., Butler, J.P. and Ingber, D.E., Mechanotransduction across the Cell Surface and

- through the Cytoskeleton, *Science*, Vol. 260, No. 5111 (1993), pp. 1124-1127.
- (10) Stamenovic, D., Ingber, D.E., Wang, N. and Fredberg, J.J., A Microstructural Approach to Cytoskeletal Mechanics based on Tensegrity, *J. Theor. Biol.*, Vol. 181, No. 2 (1996), pp. 125-136.
 - (11) Ingber, D.E., Tensegrity: The Architectural Basis of Cellular Mechanotransduction, *Annu. Rev. Physiol.*, Vol. 59 (1997), pp. 575-599.
 - (12) Ingber, D.E., Cellular Tensegrity: Defining New Rules of Biological Design that Govern the Cytoskeleton, *J. Cell. Sci.*, Vol. 104 (Pt 3) (1993), pp. 613-627.
 - (13) Wendling, S., et al., Role of Cellular Tone and Microenvironmental Conditions on Cytoskeleton Stiffness Assessed by Tensegrity Model, *Eur. Phys. J. Appl. Phys.*, Vol. 9 (2000), pp. 51-62.
 - (14) Planus, E., et al., Role of Collagenase in Mediating in vitro Alveolar Epithelial Wound Repair, *J. Cell. Sci.*, Vol. 112 (Pt 2) (1999), pp. 243-252.
 - (15) Pourati, J., et al., Is Cytoskeletal Tension a Major Determinant of Cell Deformability in Adherent Endothelial Cells?, *Am. J. Physiol.*, Vol. 274, No. 5, Pt 1 (1998), pp. C1283-1289.
 - (16) Wendling, S., Oddou, C. and Isabey, D., Stiffening Response of a Cellular Tensegrity Model, *J. Theor. Biol.*, Vol. 196, No. 3 (1999), pp. 309-325.
 - (17) Laurent, V.M., Planus, E., Fodil, R. and Isabey, D., Mechanical Assessment by Magnetocytometry of the Cytosolic and Cortical Cytoskeletal Compartments in Adherent Epithelial Cells, *Biorheology*, Vol. 40, No. 1-3 (2003), pp. 235-240.
 - (18) Maniotis, A.J., Chen, C.S. and Ingber, D.E., Demonstration of Mechanical Connections between Integrins, Cytoskeletal Filaments, and Nucleoplasm that Stabilize Nuclear Structure, *Proc. Natl. Acad. Sci. U.S.A.*, Vol. 94, No. 3 (1997), pp. 849-854.
 - (19) Albrecht-Buehler, G., Role of Cortical Tension in Fibroblast Shape and Movement, *Cell Motil Cytoskeleton*, Vol. 7, No. 1 (1987), pp. 54-67.
 - (20) Pruyne, D. and Bretscher, A., Polarization of Cell Growth in Yeast, *J. Cell. Sci.*, Vol. 113, Pt 4 (2000), pp. 571-585.
 - (21) Heidemann, S.R., Kaech, S., Buxbaum, R.E. and Matus, A., Direct Observations of the Mechanical Behaviors of the Cytoskeleton in Living Fibroblasts, *J. Cell. Biol.*, Vol. 145, No. 1 (1999), pp. 109-122.
 - (22) Hartwig, J.H. and Shevlin, P., The Architecture of Actin Filaments and the Ultrastructural Location of Actin-Binding Protein in the Periphery of Lung Macrophages, *J. Cell. Biol.*, Vol. 103, No. 3 (1986), pp. 1007-1020.
 - (23) Cheng, Y., Hartemink, C.A., Hartwig, J.H. and Dewey, C.F., Jr., Three-Dimensional Reconstruction of the Actin Cytoskeleton from Stereo Images, *J. Biomech.*, Vol. 33, No. 1 (2000), pp. 105-113.
 - (24) Hamill, O.P. and Martinac, B., Molecular Basis of Mechanotransduction in Living Cells, *Mol. Basis Mech. Living Cells*, Vol. 81, No. 2 (2001), pp. 685-740.
 - (25) Bretscher, A., Microfilament Structure and Function in the Cortical Cytoskeleton, *Annu. Rev. Cell. Biol.*, Vol. 7 (1991), pp. 337-374.
 - (26) Hochmuth, R.M., Worthy, P.R. and Evans, E.A., Red Cell Extensional Recovery and the Determination of Membrane Viscosity, *Vol. 26, No. 1* (1979), pp. 101-114.
 - (27) Laurent, V.M., et al., Assessment of Mechanical Properties of Adherent Living Cells by Bead Micromanipulation: Comparison of Magnetic Twisting Cytometry vs Optical Tweezers, *J. Biomech. Eng.*, Vol. 124, No. 4 (2002), pp. 408-421.
 - (28) Bausch, A.R., Hellerer, U., Essler, M., Aepfelbacher, M. and Sackmann, E., Rapid Stiffening of Integrin Receptor-Actin Linkages in Endothelial Cells Stimulated with Thrombin: a Magnetic Bead Microrheology Study, *Biophys. J.*, Vol. 80, No. 6 (2001), pp. 2649-2657.
 - (29) Cañadas, P., Laurent, V.M., Oddou, C., Isabey, D. and Wendling, S., A Cellular Tensegrity Model to Analyze the Structural Viscoelasticity of the Cytoskeleton, *J. Theor. Biol.*, Vol. 218, No. 2 (2002), pp. 155-173.
 - (30) Stamenovic, D. and Coughlin, M.F., A Quantitative Model of Cellular Elasticity Based on Tensegrity, *J. Biomech. Eng.*, Vol. 122 (2000), pp. 39-43.
 - (31) Wang, N., Mechanical Interactions Among Cytoskeletal Filaments, *Mech. Inter. Cyto. Filaments*, Vol. 32, No. 1 (1998), pp. 162-165.
 - (32) Laurent, V.M., et al., Tensegrity Behavior of Cortical and Cytosolic Cytoskeletal Components in Twisted Living Adherent Cells, *Acta Biotheor.*, Vol. 50, No. 4 (2002), pp. 331-356.
 - (33) Katoh, K., Kano, Y., Masuda, M., Onishi, H. and Fujiwara, K., Isolation and Contraction of the Stress Fiber, *Mol. Biol. Cell*, Vol. 9, No. 7 (1998), pp. 1919-1938.
 - (34) Katoh, K., Kano, Y., Amano, M., Kaibuchi, K. and Fujiwara, K., Stress Fiber Organization Regulated by MLCK and Rho-kinase in Cultured Human Fibroblasts, *Am. J. Physiol. Cell Physiol.*, Vol. 280, No. 6 (2001), pp. C1669-1679.
 - (35) Luby-Phelps, K., Physical Properties of Cytoplasm, *Curr. Opin. Cell. Biol.*, Vol. 6, No. 1 (1994), pp. 3-9.
 - (36) Ingber, D.E., Opposing Views on Tensegrity as a Structural Framework for Understanding Cell Mechanics, *J. Appl. Physiol.*, Vol. 89, No. 4 (2000), pp. 1663-1670.
 - (37) Glogauer, M., et al., Calcium Ions and Tyrosine Phosphorylation Interact coordinately with Actin to Regulate Cytoprotective Responses to Stretching, *J. Cell Sci.*, Vol. 110 (Pt 1) (1997), pp. 11-21.
 - (38) Evans, E. and Yeung, A., Apparent Viscosity and Cortical Tension of Blood Granulocytes Determined by Micropipet Aspiration, *Biophys. J.*, Vol. 56, No. 1 (1989), pp. 151-160.

- (39) Koffer, A., Tatham, P.E. and Gomperts, B.D., Changes in the State of Actin during the Exocytotic Reaction of Permeabilized Rat Mast Cells, *J. Cell Biol.*, Vol. 111, No. 3 (1990), pp. 919-927.
 - (40) Muallem, S., Kwiatkowska, K., Xu, X. and Yin, H.L., Actin Filament Disassembly is a Sufficient Final Trigger for Exocytosis in Nonexcitable Cells, *J. Cell Biol.*, Vol. 128, No. 4 (1995), pp. 589-598.
 - (41) Wu, Z., Wong, K., Glogauer, M., Ellen, R.P. and McCulloch, C.A., Regulation of Stretch-activated Intracellular Calcium Transients by Actin Filaments, *Biochem. Biophys. Res. Commun.*, Vol. 261, No. 2 (1999), pp. 419-425.
 - (42) Satcher, R.L.J. and Dewey, C.F.J., Theoretical Estimates of Mechanical Properties of the Endothelial Cell Cytoskeleton [see comments], *Biophys. J.*, Vol. 71, No. 1 (1996), pp. 109-118.
-