

Mechanical assessment by magnetocytometry of the cytosolic and cortical cytoskeletal compartments in adherent epithelial cells

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Abstract. This study aims at quantifying the cellular mechanical properties based on a partitioning of the cytoskeleton in a cortical and a cytosolic compartments. The mechanical response of epithelial cells obtained by magnetocytometry – a micromanipulation technique which uses twisted ferromagnetic beads specifically linked to integrin receptors – was purposely analysed using a series of two Voigt bodies. Results showed that the cortical cytoskeleton has a faster response (~ 1 s) than the cytosolic compartment (~ 30 s). Moreover, the two cytoskeletal compartments have specific mechanical properties, i.e., the cortical (resp. cytosolic) cytoskeleton has a rigidity in the range: 49–85 Pa (resp.: 74–159 Pa) and a viscosity in the range 5–14 Pa.s (resp.: 593–1534 Pa.s), depending on the level of applied stress. Depolymerising actin-filaments strongly modified these values and especially those of the cytosolic compartment. The structural relevance of this two-compartment partitioning was supported by images of F-actin structure obtained on the same cells.

1. Introduction

The cytoskeleton (CSK) of adherent cells is a complex yet organized structure, whose mechanical properties are recognized as a key determinant for cell shape and cellular function [3,5]. Different types of polymeric network and cytoskeletal organization have already been described, but their exact role and contribution in the cellular mechanical response remain to be studied [1,4]. In the present study, we basically consider the two following CSK compartments: (i) an elastic cortical CSK network (e.g., actin–ankyrin–spectrin network) associated with the plasma membrane, (ii) a tensed cytosolic CSK network comprising microfilament–microtubule–intermediate filaments that links large regions of the cytoplasm. We used a newly developed two-compartment model of the CSK response, i.e., two Voigt bodies in series, to analyze data obtained with the magnetic twisting cytometry technique, leading to a specific quantification of the mechanical properties of the cortical and cytosolic CSK networks. By principle [9], the magnetic twisting cytometry allows to apply a predetermined stress to the CSK, due to RGD-coated beads specifically linked to transmembrane integrin mechanoreceptors. Noteworthy, the proposed concept which consists of dividing the cytoskeleton into a cortical and a cytosolic compartments, not only provides a better adjustment of the mechanical response in twisted cells, but also suggests that mechanical differences (presently quantified) associated to the structural specificity (presently assessed by 3D F-actin reconstruction) of each compartment, could play a key role to understand the functional response of living adherent cells.

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2. Materials and methods

2.1. The cellular micromanipulation technique

The CSK mechanical response of adherent epithelial cells was assessed by magnetocytometry using a device similar to the one previously described by Wang et al. [9]. The original technique uses RGD-coated ferromagnetic microbeads ($4.5 \mu\text{m}$ in diameter) with a laboratory-made magnetic twisting device which allows application of controlled mechanical stresses directly to cell-surface through integrin transmembrane receptors and hence to the CSK. The bead deviation, which reflects the CSK response to mechanical stresses, was measured by an on-line magnetometer.

A549 human alveolar epithelial cells (American Type Culture Collection, Rockville, MD) were plated at a concentration of 50000 cells/well for 24 hours in bacteriologic dishes (96-well) which were previously coated with fibronectin. A 20-min incubation of RGD-coated microbeads with cells allowed to bind beads on cell-surface. A brief 0.15 T-magnetic pulse was applied to magnetize all surface bound beads in a unique direction. Then, a magnetic torque applied on the bead, was created by a much weaker vertical uniform magnetic field B_{tw} during 60 seconds ($B_{\text{tw}} \leq 6.3 \text{ mT}$). To determine the magnitude of bead rotation θ , a magnetometer continuously detected the component of the remanent magnetic field in the horizontal plane. It was assumed that the average bead deviation could be approximated by the arc-cosine of the projected magnetic field averaged over all beads ($\sim 200,000$). Typically, the time course of bead rotation magnitude shows a rapid augmentation of θ followed by a slower evolution of θ toward plateau suggesting that stability has been reached. This time course of this angular deviation was analyzed by a two-compartment rheological model, given to represent the cortical and the cytosolic CSK responses. This two-compartment analysis provided a much better curve fitting (see Fig. 1) than the previously used single compartment model [6,9–11].

2.2. Definition of the model

The two-compartment model consists of a series of two Voigt bodies representing the basic viscoelastic properties of the two CSK compartments (see Fig. 2). The CSK compartment with the fastest mechanical response, was logically assumed to be the CSK network located the closest from the bead, and was called

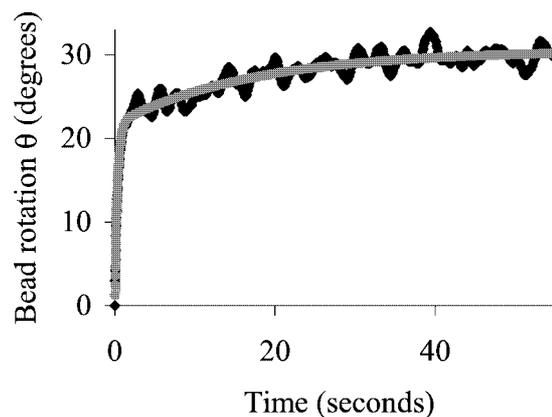


Fig. 1. Typical curve of bead rotation angle θ (in degrees) versus time (in seconds) during the application of a magnetic torque of about $1000 \text{ pN} \times \mu\text{m}$, i.e., a mechanical stress of 24 Pa in Table 1 (black curve) and the fitting curve of the theoretical equation of the two-compartment model (grey curve).

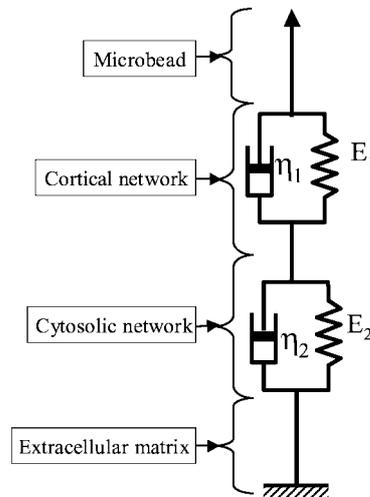


Fig. 2. Schematic representation of the rheological basis of the proposed two-compartment CSK model.

the cortical CSK compartment. By contrast, the slower CSK compartment which was logically assumed to be located the farthest from the bead, was called the cytosolic network. The choice of placing the two CSK compartments in series was guided by the observation that the local actin CSK surrounding the bead seemed to be connected to the deeper also more polymerised CSK filaments (see Results section). This choice means an infinitely rigid connection between these two CSK networks and an overall bead displacement equal to the sum of displacements for each compartment. Then, the time course of bead deviation $\theta(t)$ obtained during magnetocytometry was considered to fit the following equation:

$$\theta(t) = a_1(1 - e^{-t/\tau_1}) + a_2(1 - e^{-t/\tau_2}), \quad (1)$$

where a_1 and a_2 are the magnitudes and τ_1 and τ_2 are the time constants of the cortical and cytosolic CSK compartments, respectively. The stiffness E_1 (resp. E_2) of cortical (resp. cytosolic) CSK network was obtained from the ratio of σ_0 and a_1 (resp. a_2); σ_0 is the mechanical stress applied at the onset of the twist (equal to the ratio of initial magnetic torque modulus to bead volume). The viscosity η_1 (resp. η_2) of cortical (resp. cytosolic) CSK network was obtained from the product of the stiffness E_1 (resp. E_2) and time constant τ_1 (resp. τ_2), divided by 3.

To evaluate the effect of changing intracellular conditions on the mechanical behaviour of two CSK compartments, we measured the mechanical response in cells treated or not treated with low concentrations of cytochalasin D (1 $\mu\text{g/ml}$). Cytochalasin D is an F-actin-depolymerizing drug that we have already used in the aim of reducing internal tension in adherent cells [11].

To study the attachment of the bead to the actin cytoskeleton, we stained F-actin with fluorescent phallotoxin as previously described [11]. Then, the F-actin organization of some epithelial cells was visualized by a laser confocal microscopy using a LSM 410 microscope (Zeiss, Rueil-Malmaison, France). Optical sections were recorded every 1 μm to reveal intracellular fluorescence. The distribution of the actin cytoskeleton in the cell was revealed by a cumulative view from basal to apical optical planes. Reconstruction of F-actin contours was performed using an adapted imaging systems involving commercially available softwares (AMIRA[®] 2.1, Tgs Inc., CA and 3D-Studio Max[®]v3.1, Kinetix, CA) [2].

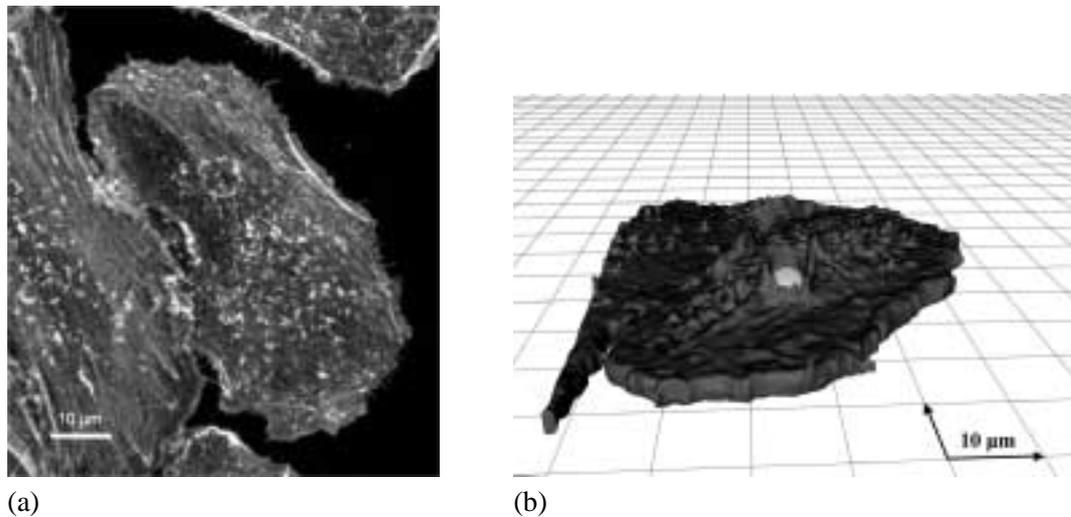


Fig. 3. Images of F-actin organization in some epithelial cells which include microbead specifically attached to integrin receptors on their apical surface. (a) Cumulative view from basal to apical optical planes obtained by confocal microscopy, (b) reconstruction of F-actin contours using optical planes.

3. Results and discussion

3.1. Structural assessment

The structural shape of the cytoskeleton both in the vicinity of attached beads and in the entire cell was characterized from the F-actin organisation. The latter was assessed by the planar optical layers describing fluorescent density. Figure 3(a) shows the cumulative view of F-actin organization from basal to apical plane. Although beads were not specifically labelled for fluorescence, the local organization of the F-actin network around every bead is clearly visible and forms an annular (or semi-spherical) structure embedding part of the bead and apparently connected to denser, i.e., more polymerising F-actin filaments, likely pertaining to the cytosolic CSK. The visualization of F-actin contours shown in Fig. 3(b) indicates that the cortical actin CSK is connected to the bead via a local annular (or semi-spherical) network on which the cytosolic network seems to be directly connected. These microscopic observations of the actin CSK tend to support the basic assumption of the model, i.e., the overall bead displacement reflects the additive and specific contribution of two compartments which are connected in series, with the cortical CSK closer from the bead than the cytosolic CSK, as shown in Fig. 2.

3.2. Mechanical assessment

The mechanical properties of the two CSK compartments were determined for four different values of mechanical stress i.e. 10, 18, 24 and 30 Pa (see Table 1). The results show that, at any applied stress, values of stiffness and viscosity related to the cytosolic CSK network were always higher than those values related to the cortical CSK. Moreover, in the range of stress values tested, the stiffness of the cytosolic CSK network increased from 74 to 159 Pa, while the stiffness of the cortical CSK network only increased from 49 to 85 Pa (see Table 1). These higher rigidity values observed for the cytosolic compartment are associated to a more pronounced stiffening response of the cytosolic CSK network compared to the cortical CSK network. These results agree with a higher degree of polymerisation of

Table 1

Mechanical properties of the two CSK compartments for 4 different mechanical stress values and assuming beads half immersed in the cytoplasm

Mechanical stress (Pa)	Cortical network			Cytosolic network		
	Stiffness (Pa)	Viscosity (Pa.s)	Time constant (s)	Stiffness (Pa)	Viscosity (Pa.s)	Time constant (s)
10	49 ± 4	5 ± 1	0.3 ± 0.1	74 ± 8*	593 ± 102*	23 ± 2*
18	60 ± 4	12 ± 1	0.7 ± 0.1	125 ± 10*	1049 ± 151*	25 ± 4*
24	64 ± 4	14 ± 1	0.7 ± 0.1	159 ± 6*	1534 ± 99*	30 ± 3*
30	85 ± 6	11 ± 1	0.4 ± 0.1	158 ± 10*	758 ± 82*	14 ± 1*

* $p < 0.05$ for difference from the cortical network value at matched mechanical stress value; results are presented as means ± SE and $n = 8$ wells for each result.

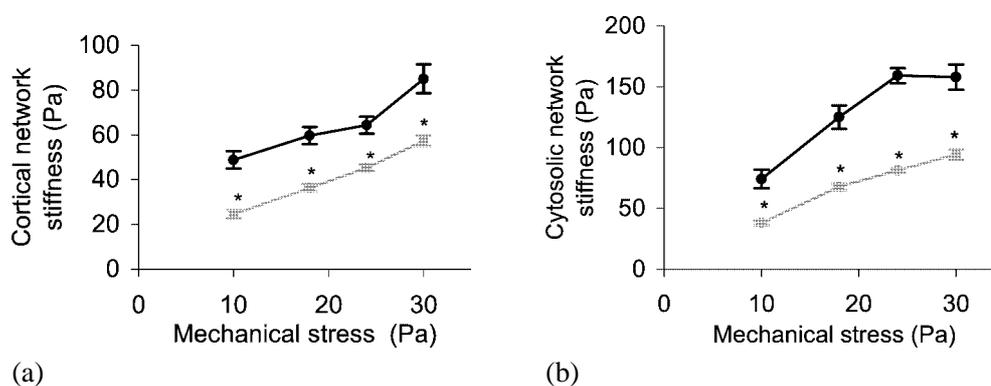


Fig. 4. Plots of the stiffness versus the applied mechanical stress for: (a) the cortical network and (b) the cytosolic network. Untreated cells correspond to black curve and cytochalasin D-treated cells correspond to grey curve. * $p < 0.05$ for difference from the value for untreated cells at matched mechanical stress value; results are presented as means ± SE and $n = 8$ wells for each result.

the cytosolic CSK network compared to the cortical. The viscosity of the cytosolic CSK network, ranges from 600 to 1600 Pa×s and was one or two orders of magnitude greater than the viscosity of the cortical CSK network, which ranged from 5 to 15 Pa×s. These results demonstrate that the two cytoskeletal compartments have very different viscoelastic behaviours. The cytosolic CSK network which is characterized by a complex and spatially interconnected networks of cross-linked filaments (actin, tubulin and intermediate filaments) appears to be a much more viscous structure likely due to a much higher number of polymeric interconnections. By contrast, the cortical CSK network (e.g., actin–ankyrin–spectrin network) associated with the plasma membrane which should not contain microtubules nor intermediate filaments while having a lesser polymerised F-actin network, has a much smaller viscosity. In summary, high rigidity and viscosity values observed for the cytosolic CSK network are consistent with the cross-linking between highly polymerised filaments, such as the actin bundles called “stress fibers” which are thought to reinforce the cell against external mechanical stresses [7,10]. The higher viscosity values of the cytosolic network could be due to the energy dissipated in friction at the junction between filaments and within the cytosol, which is known to exhibit a very high viscosity [8].

To confirm the above conclusions, we tested the effect on the mechanical properties of each compartment of a depolymerising drug (cytochalasin D) in the culture medium. For mechanical stress values of 10, 18, 24 and 30 Pa, actin filament depolymerization decreases the cytosolic CSK network stiffness by

49%, 46%, 49% and 40%, respectively, while the cortical CSK network stiffness is decreased by only 49%, 39%, 30% and 33%, respectively. These results show that, for the whole range of stress values tested, the stiffness of the treated cytosolic CSK network is considerably reduced and that this reduction is more pronounced than the one observed for the treated cortical CSK network (Fig. 4(a) and (b)). Stiffening response of the cytosolic and cortical CSK network is also reduced in these depolymerized cells (Fig. 4(a)), with however a less marked effect for the cortical CSK network (Fig. 4(b)). The present results confirm the basic assumptions of the model, i.e., the cytosolic CSK network is a highly polymerized structure while the cortical CSK network is less polymerized. In other words, for the cortical CSK network, the internal tension generated by actin filaments and stress fibers might not be the primary mechanism responsible for stiffness of the cortical CSK structure. The stability of such planar or spherical structure might indeed differ in terms of geometry and internal tension from the stability of a spatially highly tensed structure.

4. Conclusion

In conclusion, we consider that the present study, performed on adherent epithelial cells, provides convincing arguments to support the idea that the mechanical response of the cytoskeleton can be divided into a faster, softer, “fluidified” and therefore moderately polymerized cortical CSK compartment and a slower, stiffer, “solidified” and highly polymerized cytosolic CSK compartment.

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