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A Model for Stress Fiber Realignment Caused by Cytoskeletal Fluidization During Cyclic Stretching

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Abstract

Uniaxial cyclic substrate stretching results in a concerted change of cytoskeletal organization such that stress fibers (SFs) realign away from the direction of stretching. Recent experiments revealed that brief transient stretch promptly ablates cellular contractile stress by means of cytoskeletal fluidization, followed by a slow stress recovery by means of resolidification. This, in turn, suggests that fluidization, resolidification and SF realignment may be linked together during stretching. We propose a mathematical model that simulates the effects of fluidization and resolidification on cytoskeletal contractile stress in order to investigate how these phenomena affect cytoskeletal realignment in response to pure uniaxial stretching of the substrate. The model comprises of individual elastic SFs anchored at the endpoints to an elastic substrate. Employing the global stability convention, the model predicts that in response to repeated stretch–unstretch cycles, SFs tend to realign in the direction perpendicular to stretching, consistent with data from the literature. The model is used to develop a computational scheme for predicting changes in cell orientation and polarity during stretching and how they relate to the underlying alterations in the cytoskeletal organization. We conclude that depletion of cytoskeletal contractile stress by means of fluidization and subsequent stress recovery by means of resolidification may play a key role in reorganization of cytoskeletal SFs in response to uniaxial stretching of the substrate.

Keywords

Stress fiber; Fluidization; Resolidification; Pre-stress; Stability; Substrate stretching; Total potential; Contraction dipole

INTRODUCTION

It is well documented that in response to uniaxial cyclic stretching of a substrate, cytoskeletal actin stress fibers (SFs) align away from the direction of stretching.^{15,16,18,20,22,27,29–31,33} Cell contractility plays a critical role in this process. For example, inhibition of cell contractility by chemical agents causes SFs and cells to align parallel with²⁰ rather than away from stretching direction, or to slow down or completely abolish realignment.³⁰ On the other hand, recent studies have shown that cyclic stretching of the substrate causes progressive ablation of cell contractile stress by means of cytoskeletal fluidization,^{5,21,28} a stress-relieving phenomenon that characterizes behavior of a broad

range of soft materials (e.g., gels, pastes, foams, cytoskeletal lattice), but whose molecular mechanisms and origins in living cells are not well understood. Those observations appear to be at odds. For if inhibition of cell contractility by chemical agents leads to SF alignment parallel with the direction of stretching or to abolishment of realignment, then loss of contractile stress through stretch-induced fluidization should have the same effect, rather than the observed realignment away from the stretching direction. This discrepancy could be partly explained by the observation that inhibition of contractility also causes inhibition of fluidization.²⁸ Taken together, the above observations suggest that cytoskeletal realignment, fluidization and contractility are closely associated. In this study, we propose a mechanistic model that links together these phenomena. Our reasoning is as follows.

Ablation of cell contractile stress during cyclic uniaxial stretching by means of cytoskeletal fluidization results in a progressive loss of mechanical tension and mechanical stability of actin SFs. Once tension is removed from SFs, they tend to disassemble.^{6,25} New SFs would reassemble in the direction where the loss of tension due to fluidization is minimal and where mechanical stability of SFs is maintained.

Based on the above description, we propose a mathematical model of SF realignment in response to pure uniaxial stretching (i.e., where the substrate is deformed only in the stretching direction with no lateral deformations). Using a framework of elastic stability and data from the literature to describe the effect of fluidization on SF tension, we obtain predictions of SF realignment that are consistent with previously reported experimental data. The model is then used to develop a computational scheme that can simulate changes in the force contraction dipole during uniaxial substrate stretching. The contraction dipole is often used as an index of mechanical interaction between the cell and the substrate and is a metric of intracellular contractile forces that are transmitted from the cell to the substrate.^{2,3,7}

MODEL

We model individual SFs as linearly elastic line elements of length L and uniform cross-sectional area S that are anchored at the endpoints to an elastic substrate via two identical elastic focal adhesions (FAs). SFs carry initial tensile stress (prestress), which is generated by their contractile motors, before application of external loading. The prestress is transmitted to the substrate via FAs where it is opposed by substrate traction (Fig. 1). This force transfer is bidirectional; stretching of the substrate results in an increase in the traction which is transferred via FAs to SFs, thereby causing an increase in the level of stress (σ) carried by contractile SFs. Because of the symmetry of the model, we consider only one half of the SF with tensile force σS acting on its free end (Fig. 1).

We next define the total potential energy of the SF. In general, the total potential energy of an elastic system comprises two contributions: the elastic strain energy stored in the system due to deformation, and the work of the external loads on this deformation. For example, the total potential of a linear elastic spring extended to equilibrium by a constant force is the sum of the elastic energy stored in the distended spring and the potential of the applied force. Applied force potential equals the negative of the work done by the external force on the end displacement. These two contributions compete in the sense that the extension of the spring results in a restoring force which opposes the extending force. At equilibrium, these two forces have the same magnitude and opposite directions. Unlike the forces, the elastic and the force potentials are not equal, the latter being always of greater magnitude and negative, resulting in a negative total potential energy. Hence, as the applied force increases, the total potential energy of the spring-force system decreases. Accordingly, the total potential energy (Π) of the SF model (Fig. 1) is the sum of the elastic potential energy (U) stored in the SF (strain energy), and the force potential (U_f),

$$\Pi = U + U_f, \quad (1)$$

where

$$U = \frac{\sigma^2}{2E} V, \quad (2)$$

E is the elastic modulus and $V = SL$ is the volume of the SF. The force potential is equal to the negative work of the tensile force, σS , done on the displacement (u) along the axis of the SF

$$U_f = -(\sigma S) u. \quad (3)$$

At equilibrium $\partial \Pi / \partial \sigma = 0$, hence it follows from Eqs. (1)-(3) that

$$u = \frac{\sigma L}{2E}. \quad (4)$$

Combining Eqs. (1)-(4), we obtain the following expression for the total potential

$$\Pi = -\frac{\sigma^2 V}{4E}. \quad (5)$$

Stress σ in an SF that is oriented at an angle θ relative to the direction of stretching is the sum of pre-stress (σ_0) and stress ($\Delta\sigma$) induced by substrate stretching, $\sigma = \sigma_0 + \Delta\sigma$. σ_0 is generated by the SF contractile machinery, while $\Delta\sigma$ is determined by the passive elastic properties of the SF, FAs and the substrate and by strain (ε) of the SF induced by stretching, i.e., $\Delta\sigma = E\varepsilon$. Because of the substrate and FA compliance, this strain is smaller than the substrate strain ε_θ in the direction θ of the SF, i.e., $\varepsilon = \alpha\varepsilon_\theta$, where $0 \leq \alpha \leq 1$ represents the fraction of ε_θ that is transmitted to the SF. Parameter α is indicative of elastic and geometric properties of FAs and elastic properties of the substrate. If the substrate and FA stiffnesses are much greater than that of the SF, $\alpha \rightarrow 1$, whereas if they are much smaller than the SF stiffness, $\alpha \rightarrow 0$. For pure uniaxial stretching with axial substrate strain $\varepsilon_s > 0$, the linear approximation of the substrate strain ε_θ for small strain amplitudes is given as follows²⁶

$$\varepsilon_\theta = \frac{1}{2} \varepsilon_s (1 + \cos 2\theta). \quad (6)$$

We assume that σ_0 and E may change during fluidization from their baseline values, $\sigma_{0,bl}$ and E_{bl} , respectively. By normalizing Eq. (5) by the absolute value of the baseline potential,

i.e., $|\Pi_{bl}| = \sigma_{0,bl}^2 V / 4E_{bl}$, we obtain a non-dimensional potential ($\bar{\Pi}$) as follows

$$\bar{\Pi} = -\frac{E_{bl}}{E} \left(\frac{\sigma_0}{\sigma_{0,bl}} + \frac{E\alpha}{\sigma_{0,bl}} \varepsilon_\theta \right)^2. \quad (7)$$

We look for minima of $\bar{\Pi}$ over all orientations, θ , of the SF in order to identify stable configurations. To obtain a σ_0 vs. θ relationship, we utilize the data of Krishnan *et al.*²¹ as described in the next section.

FLUIDIZATION AND RESOLIDIFICATION SIMULATIONS

Krishnan and colleagues measured changes in traction distribution of cultured human airway smooth muscle cells cultured on soft polyacrylamide gel substrates (2-6 kPa stiffness) during repeated stretch pulses (i.e., a pulse-wave stretch–unstretch pattern).²¹ To quantify changes in traction, these authors used the strength of a force contraction dipole. According to the theory of elasticity, at equilibrium, traction forces at the cell-substrate interface can be replaced by a pair of opposing forces—a dipole—of the same magnitude separated by a finite distance. The action of the contraction dipole can be viewed as ‘pinching’ by which a cell probes the rigidity of the substrate. The strength of the pinching can be quantified by the dipole strength (M) which is equal to the product of the dipole force and the separation distance.^{2,7,32} Experimental data show that changes in M due to transient stretch are characterized by several distinct features. First, in response to a single stretch pulse, M decreases promptly via cytoskeletal fluidization and then recovers slowly via cytoskeletal resolidification (Fig. 2). The decrease in M is greater the greater the substrate strain amplitude (Fig. 2, inset). Second, at a given substrate strain amplitude, M decreases progressively as the number of repeated stretch pulses increases (Fig. 2). Third, between two consecutive stretch pulses, when the substrate is relaxed, part of M recuperates, more so the longer the relaxing interval is. If the relaxing interval is sufficiently long, then M can increase beyond its baseline value; the authors referred to the latter as “reinforcement” (Fig. 2). Finally, under the same experimental conditions, changes in cytoskeletal stiffness (G) mirror that of M such that when G is plotted vs. M , all data collapse to a single linear relationship $G/G_{bl} \approx M/M_{bl}$. Krishnan *et al.* also reported that FAs do not change their shape and size following a single stretch–unstretch maneuver, indicating that depletion of traction does not have an immediate effect on FA geometry.²¹

Since physical origins of cytoskeletal fluidization are not well understood, we consider two possible pathways by which cytoskeletal prestress and cytoskeletal stiffness decrease during fluidization. In the first case, stress in cytoskeletal structural elements is ablated, while their elastic properties remain unaltered. In this instance the stiffness of the whole cytoskeletal lattice would decrease because the level of cytoskeletal pre-stress is lowered. The other case is where elastic stiffness of cytoskeletal structural elements decreases which, in turn, results in a decrease in stress within structural elements. In this instance, cytoskeletal pre-stress would decrease as a result of a decrease in cytoskeletal stiffness. In both cases, one would observe that $G/G_{bl} \approx M/M_{bl}$.

Individual SFs with traction forces acting on the endpoint FAs can be viewed as force contraction dipoles. We assume that the effects of fluidization on the dipole strength that are observed at the whole cell level also take place at the level of individual SF dipoles. Since at equilibrium traction forces must balance tensile forces in the SF (Fig. 1), fluidization-induced changes in the dipole strength should be accompanied by similar changes in SF stress.

We consider in our model that the substrate is stretched uniaxially by square-wave stretch pattern with stretching and relaxing intervals of equal duration. We assume initially, before stretching, that $\sigma_{0,bl}$ is the same for all orientations of SFs. Following a stretch pulse, SFs whose orientations are more stretch-dependent will undergo greater straining and therefore, according to the data in Fig. 2 (inset), a greater decrease in prestress σ_0 than SFs that are oriented along less dependent directions. We quantify this dependence as follows. Two sets

of curves relate σ_0 to ε_θ . The first set of curves quantifies the decrease in σ_0 due to fluidization immediately following stretch pulses; we refer to these values of σ_0 as ‘fluidized’. The second set of curves determines σ_0 recovered at the end of the relaxing period due to resolidification; we refer to these values of σ_0 as the ‘recovered’. In the subsequent stretching intervals, the stress in the SF equals the sum of the recovered prestress and the elastic stress induced by ε_θ . The curve representing fluidization after the first stretch pulse is a best-fit of a third order polynomial of the experimental data from the Fig. 2 inset. The first recovery curve is also a third order polynomial obtained *ad hoc* assuming a ~65% recovery of $\sigma_{0,bl}$ at the end of the first relaxing period. Fluidization and recovery curves that correspond to subsequent maneuvers are created by decrementing the linear terms of the starting polynomials (Fig. 3a). As a result of this process, a complete ablation of the prestress in an SF can be attained after several repeated stretch–unstretch cycles. In reality, the number of cycles (n) required for complete ablation of cell contractility is much higher (e.g., our unreported observations show that $n \approx 30$ –90 four second stretch pulses repeated every 50 s are needed to completely ablate traction in endothelial cells). Since the purpose of our model is to demonstrate how fluidization affects SF realignment, we deliberately reduced the number of cycles by exaggerating the decay of σ_0 in each cycle. We also generate another set of curves that simulates the effect of reinforcement (i.e., when σ_0 is recovered beyond its baseline value). In this case, a pulse-wave stretch–unstretch pattern is applied with the relaxing intervals ~4.5 times longer than the stretching intervals. This allows σ_0 to recover to 1.1 $\sigma_{0,bl}$ at the end of the first relaxing period. Fluidization and recovery curves that correspond to subsequent maneuvers are created by incrementing the linear term of the polynomials that correspond to the first fluidization and first recovery curves (Fig. 3b).

Fluidization and resolidification are dissipative, rate of deformation-dependent processes and therefore, in general, they cannot be used in the elastic energy formulation. On the other hand, experimental data from the literature show that in response to a brief transient stretch the stress decrease due to fluidization is almost instantaneous, whereas the stress recovery due to resolidification is slow.²¹ This, in turn, suggests that the corresponding stress–strain curves shown in Figs. 3a and 3b may be approximated as pseudo-elastic and thus may be used in the potential energy formulation. We provide further justification for this approximation in the “Discussion” section.

STABILITY ANALYSIS

In search of stable configurations, we use the principle of minimum total potential energy. It asserts that a body shall deform or/and displace to a position that minimizes the total potential energy of the body, with the lost potential energy being dissipated. At equilibrium, a minimum potential energy configuration is a stable configuration. Since there can be multiple energy minima, the configuration which corresponds to the global minimum is considered the stable configuration according to the Maxwell’s criterion.¹³ In our model we assume that once the stress level in an SF reaches zero because of fluidization, the fiber disassembles. A new fiber immediately assembles in the orientation where its total potential energy corresponds to the system’s current global minimum. The lost potential energy is dissipated during SF disassembly.

With regard to the two possible pathways of fluidization discussed above, we first consider the possibility that only σ_0 changes while E remains constant at its baseline value, E_{bl} (Case I). Then we consider the possibility that E changes such that $E/E_{bl} = \sigma_0/\sigma_{0,bl}$ (Case II). Since the observed shape and size of FAs remain unaltered following a stretch–unstretch maneuver, we assume that stiffness of FAs and the substrate are not affected by fluidization

and therefore parameter α remains constant. For Case I, it follows from Eq. (7) that the non-dimensional potential (Π_I) becomes

$$\overline{\Pi}_I = -\left(\frac{\sigma}{\sigma_{0,bl}}\right)^2 = -\left(\frac{\sigma_0}{\sigma_{0,bl}} + \frac{\alpha E_{bl} \varepsilon_\theta}{\sigma_{0,bl}}\right)^2, \quad (8)$$

and for Case II, the non-dimensional potential (Π_{II}) becomes

$$\overline{\Pi}_{II} = -\frac{E_{bl}}{E} \left(\frac{\sigma}{\sigma_{0,bl}}\right)^2 = -\frac{\sigma_{0,bl}}{\sigma_0} \left(\frac{\sigma_0}{\sigma_{0,bl}} + \frac{\alpha E \varepsilon_\theta}{\sigma_{0,bl}}\right)^2. \quad (9)$$

We define $\sigma_{0,bl}/\alpha E_{bl}$ as a baseline pre-strain ($\varepsilon_{0,bl}$). Thus it follows from Eqs. (8) and (9) that

$$\overline{\Pi}_I = -\left(\frac{\sigma_0}{\sigma_{0,bl}} + \frac{\varepsilon_\theta}{\varepsilon_{0,bl}}\right)^2 \quad (10)$$

and

$$\overline{\Pi}_{II} = -\frac{\sigma_{0,bl}}{\sigma_0} \left(\frac{\sigma_0}{\sigma_{0,bl}} + \frac{E \varepsilon_\theta}{E_{bl} \varepsilon_{0,bl}}\right)^2 = -\left(\frac{\sigma_0}{\sigma_{0,bl}}\right) \left(1 + \frac{\varepsilon_\theta}{\varepsilon_{0,bl}}\right)^2. \quad (11)$$

Equations (10) and (11) are the general expressions of the total potential for Cases I and II, respectively. These expressions coincide with the total potential during stretching intervals. Note that the normalized expressions given by Eqs. (10) and (11) do not depend on α , i.e., do not explicitly depend on FA and substrate stiffnesses. However, these contributions are ‘felt’ through $\sigma_{0,bl}$ since cells that adhere to more compliant substrates tend to have lower prestress than cells that adhere to stiffer substrates.^{4,12} On the other hand, Krishnan *et al.* showed that changes of substrate stiffness from 1 to 6 kPa had virtually no effect on the normalized data for dipole strength fluidization and recovery.²¹

During relaxing intervals, where substrate strain $\varepsilon_s \equiv 0$, Eqs. (10) and (11) reduce to the corresponding ‘relaxing potential’, and Π_I and Π_{II} become quadratic and linear functions of $\sigma_0/\sigma_{0,bl}$, respectively. Since stress recovery depends on the strain amplitude and since strain in SFs depends on θ (Eq. 6), it follows that Π_I and Π_{II} during recovery also depend on θ .

Stability requires that an SF assumes the orientation that minimizes Π i.e., $\partial \Pi / \partial \theta = 0$ and $\partial^2 \Pi / \partial \theta^2 > 0$. For Case I to satisfy $\partial \Pi_I / \partial \theta = 0$, it follows from Eqs. (6) and (10) that

$$\left[\frac{\partial(\sigma_0/\sigma_{0,bl})}{\partial \varepsilon_\theta} + \frac{1}{\varepsilon_{0,bl}} \right] \sin 2\theta = 0. \quad (12)$$

Equation (12) has multiple roots corresponding to a θ of 0° , $\pm 90^\circ$, 180° , and to a θ for which the term in the square brackets vanishes. Since 0° and 180° both imply the parallel, and $\pm 90^\circ$ imply the perpendicular alignment of the SF, we only consider $\theta_1 = 0^\circ$ and $\theta_2 = 90^\circ$.

To satisfy $\partial^2 \Pi_I / \partial \theta^2|_{\theta_1} > 0$ and $\partial^2 \Pi_I / \partial \theta^2|_{\theta_2} > 0$, it follows from Eqs. (6) and (10) that for $\theta_1 = 0^\circ$,

$$\frac{\partial(\sigma_0/\sigma_{0,bl})}{\partial\varepsilon_\theta} + \frac{1}{\varepsilon_{0,bl}} > 0, \quad (13)$$

and for $\theta_2 = 90^\circ$,

$$\frac{\partial(\sigma_0/\sigma_{0,bl})}{\partial\varepsilon_\theta} + \frac{1}{\varepsilon_{0,bl}} < 0. \quad (14)$$

A third root, $0^\circ < |\theta_3| < 90^\circ$, can be obtained from the condition that the term in the square brackets in Eq. (12) vanishes. In this case, $\partial^2\Pi/\partial\theta^2|_{\theta_3} > 0$ implies that

$$\frac{\partial^2(\sigma_0/\sigma_{0,bl})}{\partial\varepsilon_\theta^2} < 0, \quad (15)$$

i.e., that the $\sigma_0/\sigma_{0,bl}$ vs. ε_θ is convex. Since curves in Fig. 3 are concave, it follows that Eq. (15) cannot be satisfied.

We consider two cases:

- a. Square-wave stretching when short stretch pulses are frequently repeated in equal intervals. In this case fluidization takes place but reinforcement does not take place, i.e., $\partial(\sigma_0/\sigma_{0,bl})/\partial\varepsilon_\theta < 0$, and therefore both Eqs. (13) and (14) may hold. Since $\partial(\sigma_0/\sigma_{0,bl})/\partial\varepsilon_\theta$ becomes increasingly negative with increasing n (Fig. 3a), the preponderance for the condition given by Eq. (14) would increase with increasing n . Thus $\theta_2 = 90^\circ$ would eventually become a stable configuration. This implies that SFs tend to assemble perpendicular to the direction of stretching.
- b. Pulse-wave stretching such that frequency of repeated stretch pulses is low enough to allow reinforcement, i.e., $\partial(\sigma_0/\sigma_{0,bl})/\partial\varepsilon_\theta > 0$. Then, Eq. (13) holds and Eq. (14) does not. Consequently, $\theta_1 = 0^\circ$ is the stable orientation and $\theta_2 = 90^\circ$ is unstable. Therefore SFs tend to assemble parallel with the direction of stretching.

During the relaxed substrate intervals ($\varepsilon_s \equiv 0$), the second term on the right-hand side of Eq. (10) vanishes. If there is no reinforcement (square-wave stretching), σ_0 would be smallest in the direction parallel to the direction of stretching and largest in the perpendicular direction where $\sigma_0 = \sigma_{0,bl}$. Consequently, Π_I would be minimum in the perpendicular and maximum in the parallel direction. In the presence of reinforcement (pulse-wave stretching), it would be quite opposite; σ_0 would be largest in the parallel direction, where $\sigma_0 > \sigma_{0,bl}$, and smallest in the perpendicular direction, where $\sigma_0 = \sigma_{0,bl}$. Consequently, Π_I would be minimum in the parallel and maximum in the perpendicular direction.

For Case II to satisfy $\partial\Pi_{II}/\partial\theta = 0$; it follows from Eqs. (6) and (11) that

$$\left[\frac{\partial(\sigma_0/\sigma_{0,bl})}{\partial\varepsilon_\theta} \left(1 + \frac{\varepsilon_\theta}{\varepsilon_{0,bl}} \right) + \frac{2}{\varepsilon_{0,bl}} \frac{\sigma_0}{\sigma_{0,bl}} \right] \sin 2\theta = 0 \quad (16)$$

Equation (16) has roots $\theta_1 = 0^\circ$, $\theta_2 = 90^\circ$, 180° , and $0^\circ < |\theta_3| < 90^\circ$, for which the term in the square brackets of Eq. (16) vanishes.

To satisfy $\partial^2 \Pi_{II} / \partial \theta^2|_{\theta_1} > 0$ and $\partial^2 \Pi_{II} / \partial \theta^2|_{\theta_2} > 0$, it follows from Eqs. (6) and (11) that for $\theta_1 = 0^\circ$,

$$\frac{\partial(\sigma_0/\sigma_{0,bl})}{\partial \varepsilon_\theta} \left(1 + \frac{\varepsilon_s}{\varepsilon_{0,bl}} \right) + \frac{2}{\varepsilon_{0,bl}} \frac{\sigma_0}{\sigma_{0,bl}} > 0, \quad (17)$$

and for $\theta_2 = 90^\circ$,

$$\frac{\partial(\sigma_0/\sigma_{0,bl})}{\partial \varepsilon_\theta} + \frac{2}{\varepsilon_{0,bl}} \frac{\sigma_0}{\sigma_{0,bl}} < 0. \quad (18)$$

Finally, for the third root, $0^\circ < |\theta_3| < 90^\circ$, $\partial^2 \Pi_{II} / \partial \theta^2|_{\theta_3} > 0$ is satisfied if

$$\frac{\partial^2(\sigma_0/\sigma_{0,bl})}{\partial \varepsilon_\theta^2} \left(1 + \frac{\varepsilon_\theta}{\varepsilon_{0,bl}} \right)^2 - \frac{6}{\varepsilon_{0,bl}^2} \frac{\sigma_0}{\sigma_{0,bl}} < 0. \quad (19)$$

Stability analysis for square- and pulse-wave stretching yields qualitatively similar results as in Case I. However, in Case II, Eq. (19) may be satisfied for some $0^\circ < |\theta_3| < 90^\circ$. This, in turn, implies that SFs may align along two symmetric oblique directions $0^\circ < \theta_3 < 90^\circ$ and $-90^\circ < \theta_3 < 0^\circ$.

In the next section, we provide numerical simulations that illustrate how the energy landscape and stability change with increasing number of stretching cycles.

NUMERICAL SIMULATIONS

We calculate the total potential energy as a function of θ for each stretching and relaxing interval. For a given θ , we calculate ε_θ (Eq. 6) and then use it to determine fluidized and recovered values of $\sigma_0/\sigma_{0,bl}$ from curves in Fig. 3. If we find that at a particular θ the fluidized value of $\sigma_0/\sigma_{0,bl} = 0$, we assume that the SF disassembles. In the subsequent stretch–unstretch cycles we do not calculate the total potential for that specific θ . Otherwise we use the recovered value of $\sigma_0/\sigma_{0,bl}$ to determine the total potential. We use $\varepsilon_s = 0.1$ and $\varepsilon_{0,bl} = 0.1$. The latter value is based on the previous observation that the baseline pre-strain in SFs of endothelial cells is $\sim 10\%$.²⁴

Case I

During first two stretching intervals, $\theta = 0^\circ$ corresponds to the energy minimum and $\theta = 90^\circ$ to the energy maximum (Fig. 4a). During the subsequent stretching intervals, $\theta = 90^\circ$ matures into a local minimum configuration while $\theta = 0^\circ$ remains the global minimum until SFs that reach a zero stress level begin to be progressively eliminated ($n \geq 5$ cycles). At that point, $\theta = 90^\circ$ becomes and remains the global minimum configuration for all subsequent cycles (Fig. 4a). During relaxing intervals, $\theta = 90^\circ$ is always the minimum and $\theta = 0^\circ$ is always the maximum energy configuration (Fig. 4b). Taken together, these results suggest that as stretching progresses, $\theta = 90^\circ$ becomes energetically the most favorable configuration and thus new SFs will assemble in the perpendicular direction.

Case II

During first three stretching intervals, $\theta = 0^\circ$ corresponds to the energy minimum and $\theta = 90^\circ$ to the energy maximum. However, at $n = 4$, both $\theta = 0^\circ$ and $\theta = 90^\circ$ are energy maxima,

whereas the minimum has shifted to $\theta \approx \pm 47^\circ$ (Fig. 4c). In the subsequent cycles, the global minimum gradually moves ($n = 5$, $\theta \approx \pm 65^\circ$; $n = 6$, $\theta \approx \pm 74^\circ$; $n = 7$, $\theta \approx \pm 84^\circ$) toward 90° as SFs that reach a zero stress level begin to be progressively eliminated ($n \geq 5$ cycles), and reaches $\theta = 90^\circ$ at $n = 8$. During relaxing intervals, $\theta = 90^\circ$ is always the minimum and $\theta = 0^\circ$ always the maximum configuration (Fig. 4d). These results are qualitatively consistent with the ones obtained for Case I, but in Case II it takes twice as many cycles for $\theta = 90^\circ$ to become a global minimum. This fact reflects the quadratic vs. linear dependence of $\bar{\Pi}$ on $\sigma_0/\sigma_{0,bl}$ in Cases I and II, respectively, as discussed above.

The results of the stability analysis are in general agreement with data from the literature that show that prolonged cyclic stretching (≥ 3 h) leads to realignment of SFs and cells perpendicular to the direction of stretching.^{18,20,22,33}

Reducing the initial SF pre-strain $\varepsilon_{0,bl}$ to 5%, it takes almost twice as many stretch–unstretch cycles for SFs to realign in the perpendicular direction than in the case of 10% pre-strain. Conversely, increasing $\varepsilon_{0,bl}$ to 15%, SFs reorient after fewer stretch–unstretch cycles than for the 10% case. Hence, our model predicts that modulating pre-strain in SFs does not prevent realignment, but rather delays it or accelerates it.

We next consider the pulse-wave stretching where the relaxing periods between two stretching maneuvers were long enough to allow stress reinforcement in SFs. Using the same algorithm and the data from Fig. 3b, we obtain the energy landscape for Cases I and II. We find that during both stretching and relaxing intervals the energy minimum is always at $\theta = 0^\circ$ and maximum at $\theta = 90^\circ$ (Fig. 5), suggesting that SFs tend to assemble parallel with the stretching direction. These data are consistent with experimental observations that cells orient parallel with stretching direction when the relaxing period between two subsequent stretch intervals was long enough (15 min) for reinforcement to take place, although in those experiments the stretch intervals was three times as long (15 min loading + 15 min constant strain + 15 min unloading) as the relaxing interval.⁹

CONTRACTION FORCE DIPOLE

To illustrate how the contraction dipole of a cell changes as a result of realignment during substrate stretching, we develop a simple computational scheme. It is based on the premise that an SF disassembles once it is released of tension and a new SF reassembles along the direction that is globally stable at that point in time. We assume that the cytoskeleton is comprised of N identical SFs attached to the substrate via FAs at its endpoints, as described above. SFs are arbitrarily oriented and are not mechanically coupled. All SFs carry the same baseline prestress and traction forces that balance this prestress form force dipoles. The strength of an individual SF dipole equals $\tau S_\tau d$, where τ is traction at the FA-substrate interface of surface area S_τ and d is the separation distance between the dipole forces (which is approximately equal to SF length L) (see Fig. 1).

Mathematically, a dipole can be represented by a matrix, which is obtained as the tensor product between the separation distance vector and the dipole force vector. For an SF oriented at an angle θ relative to the x -axis of a coordinate system whose x - and y -axes span the substrate plane, the dipole matrix $\mathbf{M}_\theta$ is given as

$$\mathbf{M}_\theta = \tau S_\tau d \begin{pmatrix} \cos^2 \theta & \sin \theta \cos \theta & 0 \\ \sin \theta \cos \theta & \sin^2 \theta & 0 \\ 0 & 0 & 0 \end{pmatrix}, \quad (20)$$

The strength of the dipole can be obtained as $M_\theta = \sqrt{\text{tr}(\mathbf{M}_\theta \mathbf{M}_\theta^T)} = \sqrt{M_{\theta,xx}^2 + 2M_{\theta,xy}^2 + M_{\theta,yy}^2}$, where $M_{\theta,ij}$ indicates components of $\mathbf{M}_\theta$. It follows from Eq. (20) that $M_\theta = \tau S_\tau d$. At equilibrium, the traction force must balance the tensile force of the SF, i.e., $\tau S_\tau = \sigma S$, and therefore the dipole strength also equals $\sigma S d$.

For a system of N identical SFs, we define the dipole matrix as a sum of individual dipole matrices given by Eq. (20). By scaling each equilibrium dipole matrix by its baseline strength, $\sigma_{0,bl} S d$, we obtain a non-dimensional dipole matrix $\mathbf{M}$ given as follows

$$\mathbf{M} = \sum_{i=1}^N \frac{\sigma_i}{\sigma_{0,bl}} \begin{pmatrix} \cos^2 \theta_i & \sin \theta_i \cos \theta_i & 0 \\ \sin \theta_i \cos \theta_i & \sin^2 \theta_i & 0 \\ 0 & 0 & 0 \end{pmatrix}. \quad (21)$$

We use Eq. (21) to predict changes in $\mathbf{M}$ during uniaxial stretching applied along the x -axis in the square-wave pattern described above. We focus on the dipole strength

$\bar{M} = \sqrt{\text{tr}(\mathbf{M} \mathbf{M}^T)}$ and on the diagonal components of $\mathbf{M}$, i.e., $\bar{M}_{xx}$ and $\bar{M}_{yy}$, which indicate the strength of the dipole ‘pinch’ along the x -axis and the y -axis, respectively. We compute $\mathbf{M}$ as follows.

We consider two different distributions of SFs before stretching. One where SFs are uniformly distributed over all orientations between 0° and 180° at 5° separation (see animations SA1 and SA2 in Supplementary Material). The other arrangement uses SFs that are uniformly distributed over a narrow range of angles centered approximately around 30° , from 13° to 48° , at 1° separation (see animations SA3 and SA4 in Supplementary Material). In both cases $N = 36$. During the simulation of square-wave stretching, if σ_0 in an SF falls to zero, it is assumed that the SF disassembles. The disassembled SF is immediately replaced by a new SF aligned in the direction that corresponds to the global energy minimum at that point in time. If the minimum corresponds to the perpendicular orientation of the SF, then its prestress equals the baseline value $\sigma_{0,bl}$. If it corresponds to an oblique orientation, we determine the corresponding prestress from ε_θ (Eq. 6) and the data in Fig. 3a. Then we compute $\mathbf{M}$ from Eq. (21) considering only the stress in the relaxing intervals and using the recovered values of the prestress. We also calculate eigenvalues and eigenvectors of $\mathbf{M}$ and use those quantities to construct ellipses whose semi-major and semi-minor axes are defined by eigenvalues and their corresponding eigenvectors. These ellipses are indicative of the changes in cell polarity and directionality.

In the case where SFs are uniformly distributed over all orientations, before stretching (i.e., $n = 0$), $\bar{M}_{xx} = \bar{M}_{yy}$ and the ellipse is a circle. Following initial stretching cycles, $\bar{M}_{xx}$, $\bar{M}_{yy}$ and $\bar{M}$ decrease with increasing n because of fluidization, while the SF distribution remains unchanged. Once SFs begin to be eliminated and replaced with new SFs, $\bar{M}_{yy}$ and $\bar{M}$ begin to increase whereas $\bar{M}_{xx}$ continues to decrease with increasing n ; $\bar{M}_{xx}$ eventually attains a saturated zero value, whereas $\bar{M}_{yy}$ and $\bar{M}$ eventually attain a saturated finite value (Fig. 6). Concomitant shape changes of the ellipses are also shown in Fig. 6 and animation SA1. In Case II, two symmetric global minima may co-exist if they correspond to oblique roots θ_3 . In such a case, the greater the number of reassembled SFs in one of the two symmetric orientations is, the more tilted from the y -axis the ellipse becomes (see animation SA2). If however, equal numbers of SFs reassemble along the two symmetric orientations, the ellipse will not be tilted. Such coexisting symmetric orientations of SFs in a single cell have been observed in the past.³⁰ Simulations in Fig. 6b correspond to the case where all reassembled SFs align along $0^\circ < \theta_3 < 90^\circ$. Comparing the shape and orientation of the ellipses between

Cases I and II, it is apparent that Case II takes more stretch–unstretch cycles for the ellipses to develop a perpendicular alignment than Case I (compare animations SA1 and SA2).

In the case where SF orientations are distributed over a narrow range centered around 30° , $\bar{M}_{xx}$ and $\bar{M}_{yy}$ are different even before stretching is applied. However, the general trend of $\bar{M}_{xx}$, $\bar{M}_{yy}$ and $\bar{M}$ with increasing n is qualitatively the same as in the case where SFs are uniformly distributed over all orientations (Fig. 7). The change in orientation of the ellipses during stretching, from the initial oblique to the final perpendicular (Fig. 7 and animations SA3 and SA4), resembles the change in directionality of living cells during uniaxial stretching.^{16,30,31}

It is noteworthy that as SFs assume the perpendicular orientation, $\bar{M}$ saturates at a value higher than the baseline value (Figs. 6, 7). The reason is that at the baseline, before the onset of stretching, individual SFs have different orientations. Thus, their net dipole strength is smaller than the sum of dipole strengths of individual SFs. However, when all SFs become aligned in the perpendicular direction, their net dipole strength equals the sum of the dipole strengths of individual SFs.

DISCUSSION

In this study, we propose a mathematical model based on elastic stability that links two phenomena that have been previously observed during cyclic uniaxial stretching of the substrate, namely realignment of SFs and loss of cell contractile stress due to cytoskeletal fluidization. These two fundamental, yet poorly understood responses of the living cell to strain are universal, and the idea that they may be mechanistically linked is novel and important. According to the model, the progressive decrease of mechanical stress in SFs by means of stretch-induced fluidization may compromise their elastic stability and lead to their disassembly. New SFs tend to assemble in the direction where the effect of fluidization is minimized, i.e., where they are stable. In the absence of a better understanding of the physical bases for the observed decrease in contractile stress during fluidization, we considered two possible pathways. Case I, where fluidization is characterized by depletion of SF stress, while their elastic properties remain unaffected. Case II, where fluidization is characterized by softening of SFs which results in reduction of their stress level. In both cases the model yielded predictions that are generally consistent with experimental data from the literature.

We also develop a computational scheme for simulating changes in the cell force contraction dipole matrix during stretching. The ellipses obtained from calculating eigenvalues and eigenvectors of the dipole matrix are independent of the coordinate system and therefore they can be a useful measure of changes in cell polarity and directionality due to changes in traction and structural organization of cytoskeletal stress fibers.

We next critically review model assumptions.

We have already pointed out that, in general, fluidization and resolidification are non-elastic processes. But because of the specific material response of cells to transient stretch, where fluidization is almost instantaneous while stress recovery is slow, we were able to use the framework of elasticity to describe stress–strain relationships that characterize those phenomena for the following reasons. On one hand, an instantaneous change in stress is associated with no energy dissipation and thus can be viewed as an elastic process. On the other hand, experimental data of Krishnan *et al.* show that stress recovery during resolidification occurs approximately linearly with the logarithm of time.²¹ This logarithmic time dependence implies that under cyclic loading, the elastic component of stress changes linearly with the logarithm of rate of deformation, whereas the dissipative component of

stress is virtually independent of the rate.¹⁷ It has been shown that if the material response is weakly dependent on the rate of deformation, then an essentially inelastic material may be conveniently described by a constitutive law of elasticity (i.e., a pseudo-strain energy function).¹¹ Therefore, the fluidized and the recovered stress–strain curves shown in Figs. 3a and 3b may be regarded as being pseudo-elastic. The good agreement between our model predictions and experimental data from the literature suggests that this approximation may be justified.

We assume that SFs whose stress is completely depleted disassemble and that new SFs assemble immediately. It has been observed in living endothelial cells that rapid shortening of SFs leads to their fast disassembly (within 5 s) and fast assembly of new SFs (within 15 s).⁶ During cyclic sinusoidal uniaxial stretching of cultured vascular smooth muscle cells SFs disassemble within 10 min from the onset of stretching and new SFs reassemble away from the stretching direction within 30 min.^{15,16} Those experiments were conducted with relatively large strain amplitudes of ~20–25%, while it has been reported that shortening below the threshold of 20% strain does not result in changes in actin polymerization.⁶ Hsu *et al.* observed no SF disassembly during equibiaxial sinusoidal stretching of endothelial cells with 10% strain amplitude.¹⁹ On the other hand, recent studies from human bladder smooth muscle cells show dramatic decrease in F-actin levels immediately after the application of only 10% equibiaxial transient stretch (4 s), and complete recovery within 5 min.⁵ Since in our model we consider periodic transient stretch maneuvers, our assumption that 10% strain amplitude would cause SF disassembly seems to be justified. The above observations also suggest that there is an apparent time lag between SF disassembly and reassembly, depending on the cell type and on stretching conditions. Incorporating this time lag into our model would not qualitatively affect model predictions. Rather, it would only cause an extended plateau in the $\bar{M}_{yy}$ vs. n relationship following fluidization before its eventual recovery, while $\bar{M}_{xx}$ vs. n relationship will not be qualitatively affected.

A key assumption in our model is that fluidization of individual SFs mirrors that of the whole cell. This is a reasonable assumption considering that SFs are main components of the contractile cytoskeleton and that observations suggest that fluidization of living cells may be coupled with their contractility.^{21,28} For example, Treppe *et al.* showed that in response to a brief transient stretch, airway smooth muscle cells treated with a contractile agonist histamine exhibited a greater decrease in stiffness due to fluidization and a faster resolidification relative to the baseline, whereas cells that were treated with relaxant DBcAMP exhibited a smaller decrease in stiffness and slower resolidification relative to the baseline.²⁸ On the other hand, when cells were treated with a myosin light chain kinase inhibitor ML7, little changes in cell's fluidization/resolidification behavior relative to the baseline was observed.²⁸

We assume that size, geometry and elastic properties of SFs and FAs do not change during stretching and therefore parameter α remains unaffected. This assumption is consistent with the observation that during initial stretch–unstretch cycles the size of FAs is not affected by fluidization and recovery.²¹ On the other hand, it has been shown earlier that under steady state conditions, FAs increase their size nearly proportionally with an increasing level of contractile stress.^{1,14} It is possible that disassembly of FAs lags behind contractile stress ablation and that SFs may disassemble before FAs do. To account for such changes in our model, new experimental data would be required.

Using the framework of elastic stability to describe SF realignment during uniaxial stretching is not novel; a number of previous models have utilized it.^{23,26,29} Common to all those models is their focus on a mechanism that reduces the level of stress in the SF during stretching. Since lowering SF stress leads to an increase in the total potential, the direction

where stress decrease is minimal is the preferential orientation for SF realignment. For example, Wang proposed a mathematical model of actin cytoskeletal reorganization in response to simple uniaxial stretching of the substrate.²⁹ Assuming that actin filaments are linearly elastic, that they carry basal elastic energy and that they disassemble when the basal energy is either lost or doubled, the model predicts that the filaments form SFs in the direction of minimal changes of their basal elastic energy. Lazopoulos and Pirentis proposed that the strain energy of SFs is non-convex, which implies that their stress-strain behavior has a region where stress decreases with increasing strain; once SFs reach this region they become unstable.²³ We recently proposed a model of SF realignment where we focused on elasticity and geometry of FAs.²⁶ More compliant FAs (i.e., the smaller the fraction of the substrate stretch transferred to the SF) would lead to SF realignment away from the direction of stretching. While the above models could describe SF realignment consistent to experimental data, those descriptions were based on either *ad hoc* assumptions regarding SF disassembly²⁹ and strain-energy,²³ or on specific geometrical and material properties of SFs and FAs.²⁶ On the other hand, in the present model, SF realignment arises from simulation of a physical process inherent to the cytoskeleton, which also provides a natural criterion for disassembly and reassembly of SFs.

Besides the potential energy minimization models, which are based on purely elastic considerations, there are models which invoke cytoskeletal viscoelasticity as a key determinant of SF realignment. In their one-dimensional model of the cell, De *et al.* proposed that in the presence of external stress, cells regulate their contractility to maintain an optimal local force in the extracellular matrix.⁸ According to their model, when applied stress rate is slow compared to the viscoelastic relaxation time constant of the cytoskeleton, the cell achieves an optimal stress level through a parallel alignment with the direction of applied stress. Conversely, when stress rate is faster than the relaxation time constant, the cell aligns perpendicular to the applied stress direction.

Hsu *et al.* observed that during harmonic cyclic uniaxial stretching at 10% strain amplitude SFs orient in the perpendicular direction when stretched at 0.1 and 1 Hz, with the alignment extent being significantly higher for 1 Hz than for 0.1 Hz; no alignment was observed at 0.01 Hz.¹⁸ To explain these observations, the authors proposed a dynamic model of SF alignment in which they incorporated two characteristic time constants, one for SF self-adjustment of their equilibrium extension (viscoelastic time constant), and the other for SF disassembly. The model predicts that when stretch rate is fast compared to the self-adjustment time constant, SFs progressively align in the perpendicular direction. However, when stretch rate is slow, there is no preferential orientation direction.

While the above models have shown that cytoskeletal viscoelasticity may be an important determinant of SF realignment, the studies of Krishnan *et al.*²¹ and Trepac *et al.*²⁸ have shown that the time course of resolidification observed in airway smooth muscle cells in response to transient stretch is markedly different from their viscoelastic power-law behavior.¹⁰ In fact, Trepac *et al.* have shown that molecular dynamics that governs resolidification is different from the one that governs stress relaxation, although they are linked together.²⁸ These authors pointed out that the transient stretch maneuver allows decoupling of fluidization and resolidification from viscoelastic relaxation such that when substrate strain is relaxed to zero, stress relaxation is almost null whereas fluidization and resolidification become fully unveiled. Since our model is based on the data from transient stretch experiments, it cannot be used to describe frequency dependence of SF realignment as observed during sinusoidal stretching, where both the viscoelastic and fluidization/resolidification properties may be important. Our model predicts that when stretching pulses are repeated at a frequency high enough not to allow stress reinforcement, SFs eventually orient perpendicular to the stretch direction. If, however, the frequency of stretching pulses

is low enough to allow reinforcement to take place, SFs align parallel with the stretching direction. Thus, our model suggests that the time scale that determines whether a cell aligns parallel or perpendicular to the direction of strain is the time scale of the transition from fluidization to reinforcement, which is a novel hypothesis of great mechanistic potential.

In summary, our model shows that depletion and recovery of cytoskeletal contractile stress by means of cytoskeletal fluidization and resolidification, respectively, may play a key role in reorganization of cytoskeletal SFs in response to uniaxial stretching of the substrate. The model is also useful for predicting changes in cell orientation and polarity during stretching and how they relate to the underlying alterations in the cytoskeletal organization. While model predictions are generally consistent with experimental data from the literature, further experiments measuring the directionality of fluidization during stretching as well as experiments where elastic and geometrical properties of SFs, FAs and the substrate can be selectively altered are needed to support the model.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

SF	Stress fiber
FA	Focal adhesion

FIGURE 1.

A free-body diagram of the model of the stress fiber (SF) of length L with two focal adhesions (FAs) at the endpoints. Because of the symmetry of the model, we consider only one half of it (black drawings). The SF carries tensile stress (σ) that is opposed by traction (τ) at the FA interface with the substrate. The pair of traction forces exerted on the substrate creates the force contraction dipole with d indicating the separation distance between the forces.

FIGURE 2.

Change in the contractile dipole strength vs. time relationship as a result of repeated stretch pulses redrawn from the experimental data of Krishnan *et al.*²¹ The dipole strength (M) is normalized by its baseline value (M_{bl}) indicated by the thin dashed line; time (t) is normalized by the stretching time interval (t_{str}) indicated by gray columns. The heavy solid line indicates the time course of M/M_{bl} which decreases promptly following each stretch interval and then recovers during the relaxing interval. Open circles correspond to fluidized values of M and solid circles correspond to recovered values of M at the end of relaxing. If the relaxing interval is sufficiently long, M/M_{bl} will recover beyond the baseline value (reinforcement) as indicated by the heavy dotted line. Inset: M/M_{bl} decreases with increasing substrate strain amplitude. Dots are experimental data and the solid line is the best fit of a cubic polynomial.

FIGURE 3.

The change in stress fiber prestress (σ_0) vs. strain (ϵ_θ) in the substrate in the direction θ of a stress fiber during fluidization (red) and recovery (blue). σ_0 is normalized by its baseline value ($\sigma_{0,bl}$). The heavy red line corresponds to the curve in Fig. 2 inset. In the case where σ_0 is not completely recovered during relaxing (a), the heavy blue line represents the recovery curve during the first relaxing interval. In the case where reinforcement takes place (b), the heavy red line corresponds to the best fit cubic polynomial from Fig. 2 inset (i.e., $\sigma_0/\sigma_{0,bl} = 1 - 1.51\epsilon_\theta + 140\epsilon_\theta^2 - 587\epsilon_\theta^3$; $r^2 = 1$), while the heavy blue line indicates the reinforcement curve during the first relaxing interval. The rest of the red and blue curves correspond to subsequent stretch pulses as described in the text.

FIGURE 4.

Changes in the total potential energy landscape as a function of orientation (θ) of stress fibers and different numbers (n) of stretch cycles in the case where fiber stress does not fully recover after fluidization. Π_I is the normalized potential for Case I during stretching (a) and relaxing (b) intervals. Π_{II} is the normalized potential for Case II during stretching (c) and relaxing (d) intervals. Where energy curves do not span the whole range of angles, stress fibers have reached zero stress level for the corresponding orientations and are subsequently eliminated.

FIGURE 5.

Changes in the total potential energy level as a function of orientation (θ) of stress fibers for different numbers (n) of stretching cycles in the case where reinforcement takes place. Π_I is the normalized potential for Case I during stretching (a) and relaxing (b) intervals. Π_{II} is the normalized potential for Case II during stretching (c) and relaxing (d) intervals.

FIGURE 6.

Strength ($\bar{M}$) of the normalized force contraction dipole and $\bar{M}_{xx}$ and $\bar{M}_{yy}$ components of the dipole matrix (Eq. 21) vs. the number (n) of stretching cycles. Stress fibers are uniformly distributed over all orientations for Case I (a) and Case II (b). The ellipses indicate how polarity and orientation of the dipole matrix change during stretching; each ellipse corresponds to different n , with the left ellipse corresponding to $n = 0$ (before stretching). The double-headed arrow indicates the direction of substrate stretching.

FIGURE 7.

Strength ($\bar{M}$) of the normalized force contraction dipole and $\bar{M}_{xx}$ and $\bar{M}_{yy}$ components of the dipole matrix (Eq. 21) vs. the number (n) of stretching cycles. Stress fibers are uniformly distributed over a limited range of angles around $\sim 30^\circ$ for Case I (a) and Case II (b). The ellipses indicate how polarity and orientation of the dipole matrix change during stretching; each ellipse corresponds to different n , with the left ellipse corresponding to $n = 0$ (before stretching). The double-headed arrow indicates the direction of substrate stretching.