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Mechanical Stability Determines Stress Fiber and Focal Adhesion Orientation

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Abstract

It is well documented in a variety of adherent cell types that in response to anisotropic signals from the microenvironment cells alter their cytoskeletal organization. Previous theoretical studies of these phenomena were focused primarily on the elasticity of cytoskeletal actin stress fibers (SFs) and of the substrate while the contribution of focal adhesions (FAs) through which the cytoskeleton is linked to the external environment has not been considered. Here we propose a mathematical model comprised of a single linearly elastic SF and two identical linearly elastic FAs of a finite size at the endpoints of the SF to investigate cytoskeletal realignment in response to uniaxial stretching of the substrate. The model also includes the contribution of the chemical potential energies of the SF and the FAs to the total potential energy of the SF–FA assembly. Using the global (Maxwell's) stability criterion, we predict stable configurations of the SF–FA assembly in response to substrate stretching. Model predictions obtained for physiologically feasible values of model parameters are consistent with experimental data from the literature. The model shows that elasticity of SFs alone can not predict their realignment during substrate stretching and that geometrical and elastic properties of SFs and FAs need to be included.

Keywords

Stress fiber; Focal adhesions; Cytoskeleton; Stability; Substrate stretching; Potential; Elasticity

Introduction

The mechanical environment of the adherent cells influences their form and function. Very often, these mechanical stimuli are non-uniform, both *in vivo* and in laboratory conditions. It is well documented in a variety of adherent cell types that in response to anisotropic stretching of the substrate, cells alter their cytoskeletal organization.^{4,11,14,15,18,22,25,28–30,33} For example, in response to pure uniaxial cyclic stretching¹ of two-dimensional cell cultures, the

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¹Pure uniaxial stretching corresponds to the case where cells are deformed in the direction of substrate stretching and not in the perpendicular direction, as opposed to the simple uniaxial stretching where the cells are deformed in both the direction of stretching and in the perpendicular direction due to the Poisson's effect.

actin cytoskeleton (CSK) of adhering cells remodels such that actin stress fibers (SFs) and focal adhesions (FAs) align perpendicular to the stretching direction.^{15,31,33} It has been shown that stretch-induced rearrangements of SFs and FAs are a result of the interplay between Rho pathway activity and the extent of cell stretching.¹⁵ While Rho activities facilitate remodeling of SFs and FAs and also modulate cytoskeletal contractile stress and hence permit the reorganization of the CSK, Rho activities are not directional and thus cannot explain alone why SFs align in a particular direction in response to stretching. Thus, while biochemical factors certainly influence SF orientation, we do not fully understand what factors determine the directionality of SF orientation.

In 2000, Wang²⁹ proposed a mathematical model of actin cytoskeletal reorganization in response to uniaxial stretching of the substrate. 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. For simple uniaxial stretching of the substrate, the model predicts that SFs align at $\sim 60^\circ$ relative to the direction of stretching, consistent with experimental observations.²⁹ According to the model, this angle of orientation depends on the Poisson's ratio of the substrate. In the case of substrates of zero Poisson's ratio, which corresponds effectively to the pure uniaxial stretching, the model predicts that SFs align perpendicular to the direction of stretching, which is also consistent with the experimental observations.^{15,31,33} Lazopoulos and Pirentis¹⁹ studied SF realignment in response to substrate stretching as an elastic stability phenomenon. They proposed that SFs would align in the direction where their potential energy would attain a global minimum which, according to Maxwell's criterion of stability, represents a stable configuration.⁷ This model predicts that during pure uniaxial stretching a stable configuration of SFs is perpendicular to the direction of stretching. However, to obtain such a prediction, it was necessary to assume a specific class of non-linear constitutive laws for SFs—Mooney–Rivlin materials with non-convex strain energy function—whereas experimental measurements on isolated SFs show that they behave as a convex elastic material.⁶ Experimental data also show that SFs exhibit notable nonlinear behavior only at high strains ($> 40\%$), while substrate strains applied to the cells usually do not exceed 20% below which SFs behave as a linearly elastic material.⁶ Importantly, none of the above models considered the contribution of FAs to cytoskeletal realignments in response to stretching of the substrate, notwithstanding that SFs are physically linked to the substrate via FAs, whose mechanical properties may play an important role in the overall stability of the CSK.

Here we propose a mathematical model of an individual SF–FA assembly to study its alignment in response to uniaxial stretching of the substrate. Using the global stability criterion, the model predicts alignments of the SF–FA assembly during a simple and a pure uniaxial stretching that are consistent with experimental data from the literature, without the need for a non-convex strain energy function. The model shows that elasticity of SFs alone can not predict their alignment in response to substrate stretching that is consistent with experimental data from the literature, and that geometrical and elastic properties of SFs and FAs need to be included.

Model Formulation

Within living cells, SFs carry initial contractile stress (prestress), before application of external loading.⁶ This prestress is primarily caused by myosin motor proteins that are capable of generating tensile force in the actin filaments through the ATP-driven process of crossbridge cycling. 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, causing thereby an increase in the level of stress carried by contractile SFs. It has been shown experimentally that the prestress

causes a pre-extension or pre-strain of SFs; i.e., in the presence of the prestress, SFs are elongated, more so the greater the prestress.^{3,21}

We model individual SFs as elastic line elements that are anchored at the endpoints to the substrate via two identical elastic FAs. We assume a one-dimensional state of stress and strain in the SF–FA assembly in the direction of the SF long axis. Therefore, the SF and FAs can only change their lengths, while the diameter of the SF and the width and the height of the FAs remain unchanged. The SF carries uniform tensile prestress (σ), which is transmitted to the substrate via the FAs, where it is opposed by traction. This pulling action of σ also generates stress within the FAs which together with the traction opposes σ (Fig. 1). In response to substrate stretching, SFs and FAs reassemble as they realign. This process depends on the extent of the mechanical stress that SFs and FAs carry¹⁵ since the stress lowers their chemical potential and thus increases their ability to remodel.

The total potential (U) of the SF–FA assembly is the sum of the elastic, chemical, and loading potentials, given as follows

$$U = U_{\text{el}}^{\text{SF}} + 2U_{\text{el}}^{\text{FA}} + U_{\text{chem}}^{\text{SF}} + 2U_{\text{chem}}^{\text{FA}} + U\sigma, \quad (1)$$

where $U_{\text{el}}^{\text{SF}}$ and $U_{\text{el}}^{\text{FA}}$ are the elastic potential energies and $U_{\text{chem}}^{\text{SF}}$ and $U_{\text{chem}}^{\text{FA}}$ are the total chemical potential energies of the SF and the FAs, respectively, and $U\sigma$ is the loading potential of σ ; factor 2 in Eq. (1) indicates that there are two identical FAs in the SF–FA assembly. We do not include in Eq. (1) the contribution of the elastic potential of the substrate. The reason is that in substrate stretching experiments, the stiffness of the substrate is much greater than the stiffness of both SFs and FAs and thus the cell-induced traction produces negligible strain in the substrate. We assume that the SF and the FAs are linearly elastic (Hookean), i.e.,

$$(a) \quad U_{\text{el}}^{\text{SF}} = \frac{1}{2} V_0^{\text{SF}} E_{\text{SF}} \varepsilon^2 \quad \text{and} \quad (b) \quad U_{\text{el}}^{\text{FA}} = \frac{\tau^2}{2E_{\text{FA}}} V_0^{\text{FA}}, \quad (2)$$

where V_0^{SF} , E_{SF} , and ε are the initial volume, the elastic modulus, and strain of the SF, respectively; V_0^{FA} , E_{FA} , and τ are the initial volume, the elastic modulus, and the average stress of the FAs, respectively.

The chemical potential is defined as the change in the free energy due to a change in the number of molecules in the system when other thermodynamic quantities are kept constant. In the absence of mechanical stress, the chemical potential of aggregated molecules is referred to as the standard chemical potential. In the presence of mechanical stress, the chemical potential of the aggregate decreases. The total chemical potential energy of a molecular aggregate equals the product of the number of aggregated molecules times the standard chemical potential reduced by a term that depends on the stress. Thus, $U_{\text{chem}}^{\text{SF}}$ and $U_{\text{chem}}^{\text{FA}}$ can be written as follows^{12,24}

$$(a) \quad U_{\text{chem}}^{\text{SF}} = N_{\text{SF}} \mu_0^{\text{SF}} - \sigma V_0^{\text{SF}} \quad \text{and} \quad (b) \quad U_{\text{chem}}^{\text{FA}} = N_{\text{FA}} \mu_0^{\text{FA}} - \tau V_0^{\text{FA}}, \quad (3)$$

where N_{SF} and N_{FA} are the number of aggregated molecules and μ_0^{SF} and μ_0^{FA} are the standard chemical potential in the SF and the FAs, respectively.

The potential due to σ is given as

$$U\sigma = -V_0^{\text{SF}}\sigma u_n, \quad (4)$$

where u_n is the displacement gradient of the SF along its axis.

For simplicity and mathematical transparency, we consider the linear strain approximation, i.e., when $\varepsilon = u_n$. Combining Eqs. (1)–(4), we obtain that

$$U = \frac{1}{2}V_0^{\text{SF}}E_{\text{SF}}u_n^2 + \frac{\tau^2}{E_{\text{FA}}}V_0^{\text{FA}} + N_{\text{SF}}\mu_0^{\text{SF}} - \sigma V_0^{\text{SF}} + 2N_{\text{FA}}\mu_0^{\text{FA}} - 2\tau V_0^{\text{FA}} - V_0^{\text{SF}}\sigma u_n. \quad (5)$$

To obtain a relationship between σ and τ , we assume that σ is transferred from the SF to the FA over its length L_{FA} , from the proximal end of the FA ($\zeta = L_{\text{FA}}$) to its distal end ($\zeta = 0$), such that at $\zeta = 0$ the stress in the FA equals T and at $\zeta = L_{\text{FA}}$, the stress equals σ (Fig. 1).²⁴ Based on this description, we obtain (see Appendix for the derivation) that the average stress in the FAs is

$$\tau = \frac{1}{2} \left(1 - 2 \frac{A_{\text{SF}}}{A_{\text{FA}}} \right) \sigma. \quad (6)$$

By substituting Eq. (6) into Eq. (5), we obtain that

$$U = N_{\text{SF}}\mu_0^{\text{SF}} + 2N_{\text{FA}}\mu_0^{\text{FA}} + V_0^{\text{SF}} \left[\frac{1}{2}E_{\text{SF}}u_n^2 - \sigma u_n - (1+\alpha)\sigma + \beta \frac{\sigma^2}{2E_{\text{SF}}} \right], \quad (7)$$

where

$$\begin{aligned} \text{(a) } \alpha &= \left(\frac{A_{\text{FA}}}{A_{\text{SF}}} - 2 \right) \frac{h_{\text{FA}}}{L_{\text{SF}}} \text{ and} \\ \text{(b) } \beta &= \frac{1}{2} \frac{E_{\text{SF}}}{E_{\text{FA}}} \left(1 - 2 \frac{A_{\text{SF}}}{A_{\text{FA}}} \right)^2 \frac{A_{\text{FA}} h_{\text{FA}}}{A_{\text{SF}} L_{\text{SF}}}, \end{aligned} \quad (8)$$

$h_{\text{FA}} = V_0^{\text{FA}}/A_{\text{FA}}$ is the average thickness of the FAs and $L_{\text{SF}} = V_0^{\text{SF}}/A_{\text{SF}}$ is the length of the SF (Fig. 1). The non-dimensional parameters α and β represent the contributions of the FAs to the stress-dependent part of the chemical energy and to the elastic potential of the SF–FA assembly, respectively, relative to the corresponding contributions of the SF. It is noteworthy that α depends only on the geometrical properties of the SF and the FAs, whereas β depends on both geometrical and elastic properties of the SF and the FAs.

Since U attains minimum or maximum at equilibrium, $\partial U/\partial u_n = 0$, and it follows from Eq. (7) that $\partial U/\partial u_n = V_0^{\text{SF}} (E_{\text{SF}} u_n - \sigma) = 0$, which implies Hook's law, i.e., $\sigma = E_{\text{SF}} u_n$. By substituting this into Eq. (7), we obtain that in equilibrium

$$U = N_{\text{SF}} \mu_0^{\text{SF}} + 2N_{\text{FA}} \mu_0^{\text{FA}} - V_0^{\text{SF}} E_{\text{SF}} \left[(1+\alpha) u_n + (1-\beta) \frac{u_n^2}{2} \right]. \quad (9)$$

Stability Analysis

We use Eq. (9) to analyze the mechanical stability of the SF-FA assembly for the case of uniaxial stretching of the substrate. To determine the globally stable configuration of the SF-FA assembly, we apply Maxwell's stability criterion.¹⁹

Suppose the SF-FA assembly is anchored to a substrate and that it is oriented at an angle θ relative to the direction of substrate stretching. The x -axis of the Oxy coordinate system that lies in the substrate plane is set to be parallel with the direction of stretching (Fig. 2). The pre-strain in the SF prior to substrate stretching is given by the initial displacement gradient $u_0 \geq 0$. The deformation of the substrate due to uniaxial stretching is given in terms of the displacement gradient $u_x \geq 0$ along the x -axis. This deformation further extends the SF. For small (linear) deformations, the net displacement gradient, u_n , of the SF is given as follows

$$u_n = u_0 + \frac{1}{2} u_x [(1-\nu) + (1+\nu) \cos 2\theta], \quad (10)$$

where $-1 \leq \nu \leq 0.5$ is the Poisson's ratio of the substrate. (Derivation of Eq. (10) is given in Appendix.) By substituting Eq. (10) into Eq. (9), we obtain U as a function of θ . Stability requires that the SF-FA assembly assumes a configuration that minimizes U , i.e., $\partial U/\partial \theta = 0$ and $\partial^2 U/\partial \theta^2 > 0$. In general, there are more than one local minima (stable configurations), and hence, according to the Maxwell's criterion, we seek the orientation θ that renders U a global minimum. We also assume that $N_{\text{SF}} \mu_0^{\text{SF}}$ and $N_{\text{FA}} \mu_0^{\text{FA}}$ are independent of θ and thus, for the purpose of the stability analysis, we regarded them as constants. According to Eq. (9), $\partial U/\partial \theta = 0$ implies that

$$[1 + \alpha + (1 - \beta) u_n] \frac{\partial u_n}{\partial \theta} = 0. \quad (11)$$

From Eq. (11), it follows that U may have multiple minima, i.e., when $\partial u_n/\partial \theta = 0$ and when $u_n = (1 + \alpha)/(\beta - 1)$. Since u_0 and u_x do not depend on θ , it follows from Eq. (10) that $\partial u_n/\partial \theta = 0$ is satisfied for $\theta_1 = 0^\circ$ and $\theta_2 = 90^\circ$, i.e., the orientation parallel with and perpendicular to the direction of stretching, respectively (note that we consider the orientations $\theta = \pm 180^\circ$ and $\theta = -90^\circ$ as equivalent to $\theta = 0^\circ$ and $\theta = 90^\circ$, respectively). A third root of Eq. (11) is obtained from the condition $u_n = (1 + \alpha)/(\beta - 1)$ and Eq. (10), i.e.,

$$\theta_3 = \pm \frac{1}{2} \arccos \left[2 \frac{1+\alpha}{\beta-1} \frac{1}{u_x (1+\nu)} - \frac{2u_0 + u_x (1-\nu)}{u_x (1+\nu)} \right]. \quad (12)$$

According to Eq. (10), $\partial^2 u_n / \partial \theta^2|_{0^\circ} < 0$ and $\partial^2 u_n / \partial \theta^2|_{90^\circ} > 0$ and therefore it follows from Eq. (9) that $\partial^2 U / \partial \theta^2|_{0^\circ} > 0$ if

$$1 + \alpha + (1 - \beta)(u_0 + u_x) > 0 \quad (13)$$

and $\partial^2 U / \partial \theta^2|_{90^\circ} > 0$ if

$$1 + \alpha + (1 - \beta)(u_0 - \nu u_x) < 0. \quad (14)$$

If, however, $u_n = (1 + \alpha)/(\beta - 1)$, then the corresponding $\partial^2 U / \partial \theta^2|_{\theta_3} > 0$ if

$$\beta > 1. \quad (15)$$

Based on the above, the local minima of U are determined by α (geometrical properties of FAs and SFs), β (elastic and geometrical properties of FAs and SFs), u_0 (SF pre-strain), u_x (substrate extension), and ν (Poisson's ratio of the substrate). To illustrate how the different components and potentials of the SF-FA assembly contribute to stability, we consider the following cases.

- a. The SF is anchored to the substrate via rigid pins so that the FAs do not contribute and the chemical energies are not taken into account, i.e., only $U_{\text{el}}^{\text{SF}}$ and U_σ contribute to the total potential U . In this case, the first two and the last two terms on the right-hand side of Eq. (7) are dropped out and the conditions given by Eqs. (13) and (14) become $u_0 + u_x > 0$ and $u_0 - \nu u_x < 0$, respectively, whereas Eq. (15) does not apply. Since $u_0 \geq 0$ and $u_x \geq 0$, the first inequality is satisfied except in the trivial case when $u_0 = u_x = 0$, whereas the second inequality may hold only for very small values of u_0 provided $\nu > 0$. Thus, U may have local minima at both $\theta = 0^\circ$ and $\theta = 90^\circ$. However, based on Eqs. (9) and (10), it is easy to show that the global minimum always corresponds to $\theta = 0^\circ$, i.e., the SF would tend to align parallel with the direction of stretching.
- b. The SF is anchored to the substrate via elastic FAs of a finite size, but the chemical energies are not taken into account, i.e., only $U_{\text{el}}^{\text{SF}}$, $U_{\text{el}}^{\text{FA}}$ and U_σ contribute to U . In this case, the first two and the fifth terms on the right-hand side of Eq. (7) are dropped out and Eqs. (13)–(15) become $(1 - \beta)(u_0 + u_x) > 0$, $(1 - \beta)(u_0 - \nu u_x) < 0$ and $\beta > 1$, respectively. If $\beta < 1$, then the first inequality is satisfied except in the trivial case; the second inequality is satisfied provided $u_0 < \nu u_x$ and $\nu > 0$; and the third inequality is not satisfied. Thus we have the same outcome as in case (a), i.e., alignment parallel with the direction of stretching. If $\beta > 1$, the first inequality is not satisfied; the second inequality is satisfied provided $u_0 > \nu u_x$; and the third inequality is satisfied. However, the global minimum of U corresponds to $\theta = 90^\circ$, i.e., alignment perpendicular to the direction of stretching. On the other hand, if $\beta > 1$ and $u_0 < \nu u_x$, then only the third inequality is satisfied and the global minimum corresponds to $(1/2)\text{arc-cos}[(1 - \nu)/(1 + \nu)] < \theta < 90^\circ$, i.e., an oblique alignment. An illustrative example of the dependence of θ that corresponds to the global minimum of U for a wide ranges of combinations of $A_{\text{FA}}/A_{\text{SF}}$ and $h_{\text{FA}}/L_{\text{SF}}$ and for $E_{\text{SF}}/E_{\text{FA}} = 150$, $u_0 = 0.1$, $u_x = 0.1$, and $\nu = 0.35$ is shown in Fig. 3. The range of the parameters is determined based on experimental data which are discussed below. These graphs show that the global minimum

corresponds to either $\theta = 0^\circ$ or $\theta = 90^\circ$. Additional results obtained for $E_{\text{SF}}/E_{\text{FA}} = 15$ and $E_{\text{SF}}/E_{\text{FA}} = 1500$ are given in Supplementary Material.

- c. By including the chemical energies $U_{\text{chem}}^{\text{SF}}$ and $U_{\text{chem}}^{\text{FA}}$ to the total potential from the case (b), we obtain the stability conditions given by Eqs. (13)–(15). Since $U_{\text{chem}}^{\text{SF}}$ and $U_{\text{chem}}^{\text{FA}}$ decrease with increasing stress (Eq. 3), the energetically favorable alignment during substrate stretching should be parallel with the direction of stretching. On the other hand, for a wide range of parameter values the elastic potential of the SF–FA assembly favors the perpendicular alignment, as shown in Fig. 3. Thus, these competing influences of the elastic and the chemical potentials could yield the global minimum of U that corresponds to $0^\circ \leq \theta \leq 90^\circ$. To illustrate this, we calculate θ for the same range of parameters as in Fig. 3. While the global minima that correspond to either $\theta = 0^\circ$ or $\theta = 90^\circ$ have the same pattern as in Fig. 3, there exists now a range of parameter values for which the global minima correspond to angles $0^\circ < \theta < 90^\circ$ (Fig. 4a). The probability density of θ computed from all values in Fig. 4a is shown in Fig. 4b. Additional results obtained for $E_{\text{SF}}/E_{\text{FA}} = 15$ and $E_{\text{SF}}/E_{\text{FA}} = 1500$ are given in Supplementary Material.

The above results are novel. First, they show that within the framework of linear elasticity, the contribution of elastic and geometrical properties of SFs alone are not sufficient for predicting SF alignments which are consistent with experimental data. For that, the contributions of elastic and geometrical properties of FAs also need to be included. Second, the results show that the contribution of the stress-dependent parts of the chemical energies allows for oblique alignments which are experimentally observed.^{28,29} These contributions of the FAs and of the chemical energies were not considered in previous models of SF alignment.^{13,19,29,32}

We further illustrate the above stability analysis with numerical simulations of cyclic substrate stretching using parameter values obtained from experimental data from the literature.

Numerical Simulations

We first calculate values for parameters α and β . The diameter of actin SFs of living cells ranges from several hundred nanometers to $1 \mu\text{m}$, whereas their length $L_{\text{SF}} \geq 20 \mu\text{m}$.^{3,21} Here we assume that the SF diameter is 300 nm , which yields $A_{\text{SF}} = 0.071 \mu\text{m}^2$, and that $L_{\text{SF}} = 20 \mu\text{m}$. The Young's modulus of the SF is $E_{\text{SF}} = 1.5 \text{ MPa}$, based on the data from the tensile test of isolated actin SFs.⁶ The interfacial area of the FAs, A_{FA} , with the substrate can vary from 1 up to $10 \mu\text{m}^2$. We chose $A_{\text{FA}} = 3 \mu\text{m}^2$ based on experimental data of Balaban *et al.*¹ Measured average thickness of FAs is $h_{\text{FA}} \approx 100 \text{ nm}$.⁸ The elastic modulus E_{FA} of FAs is estimated from data from magnetic pulling experiments of surface integrin receptors.^{2,23} Measurements on cultured fibroblasts yielded highly scattered values of E_{FA} , from 10^0 to 10^3 kPa , with an average value of $E_{\text{FA}} = 20\text{--}40 \text{ kPa}$.² Measurements on cultured endothelial cells yielded $E_{\text{FA}} \approx 2 \text{ kPa}$.²³ In the absence of more precise measurements, we chose ad hoc $E_{\text{FA}} = 10 \text{ kPa}$. Based on the above values, we obtain from Eq. 8a,b that $\alpha = 0.2$ and $\beta = 14.4$.

Studies on living endothelial cells have shown that their SFs carry pre-strain u_0 ranging from ~ 0.05 to 0.3 , depending on the level of cell contractility.^{3,21} The higher values of u_0 were obtained from stimulated cells and the lower values were obtained from cells in which the contractile force generation was inhibited, with a baseline value of 0.1 .²¹

During uniaxial cyclic stretching of the substrate along the x -axis, the substrate displacement gradient u_x varies between zero and a finite value as follows

$$u_x = u_m (1 - \cos \omega t), \quad (16)$$

where u_m is the amplitude and ω is the frequency of stretching, and t varies from 0 to P , where P is the period of a cycle. Since our model is elastic and does not include inertia, there are no effects of ω . Thus, we consider only a single cycle, i.e., $0^\circ \leq \omega t \leq 360^\circ$. In many experimental studies, the peak-to-peak substrate strain varies between 0 and 0.2,^{11,15,25,31,33} i.e., u_m varies between 0 and 0.1. By combining Eqs. (9), (10), and (16), we obtain a relationship of U vs. θ during a single stretching cycle of the substrate. From this relationship, we then obtain configurations (i.e., θ) of the SF-FA assembly, at which U attains a global minimum.

We first consider simple uniaxial stretching. For the baseline value of the pre-strain of $u_0 = 0.1$, peak substrate strain of $(u_x)_{\max} = 0.1$ (i.e., $u_m = 0.05$), and $\nu = 0.35$, the model predicts that throughout the stretching cycle there are multiple minima that occur between $\pm 65^\circ$ and $\pm 71^\circ$ and at $\pm 90^\circ$ (Fig. 5). However, multiple global minima are found only between $\pm 65^\circ$ and $\pm 71^\circ$. Thus, these results suggest that SFs and FAs tend to orient at angles ranging from $\pm 65^\circ$ to $\pm 71^\circ$ with respect to the direction of substrate stretching. This is not far from the experimentally observed SF orientations of around $\pm 60^\circ$ – 70° reported in the literature.^{28,29} The reason for the multiple minima during a stretching cycle is that the substrate strain, u_x , changes during the cycle (Eq. 16). We have shown above, in section “Stability Analysis,” that increasing u_x may shift the minimum of U toward lower angles θ (Eq. 12).

For pure uniaxial stretching, we set the Poisson's ratio $\nu = 0$. In this case, the condition given by Eq. (14) becomes $u_0 > (1 + \alpha)/(\beta - 1)$, i.e., stability in the perpendicular direction is entirely determined by the pre-strain and by the geometrical and elastic properties of the SF and FAs, regardless of the extent of substrate stretching. If this condition is not satisfied, then the SF-FA system will align either in the direction of stretching or at some oblique angle $0^\circ < \theta < 90^\circ$, depending on the properties of the SF and FAs as well as on the substrate strain u_x . Using the same parameter values as above, we obtained simulations for the baseline pre-strain $u_0 = 0.1$ and for $(u_x)_{\max} = 0.1$. Since in this case $u_0 > (1 + \alpha)/(\beta - 1)$ is satisfied, the model predicts that throughout the stretching cycle the global minimum corresponds to $\theta = \pm 90^\circ$ (Fig. 6). Thus, the SFs and FAs would orient perpendicular to the direction of stretching. This is consistent with the experimental data from the literature.¹⁵ We next consider the situation when the pre-strain is reduced to the level that corresponds to the case where the contractile force generation is inhibited, i.e., $u_0 = 0.05$.²¹ In this case $u_0 < (1 + \alpha)/(\beta - 1)$ and therefore, the SF-FA assembly can not orient in the perpendicular direction. The model predicts that during the initial part of the stretching cycle, the local minima correspond to 0° , but approaching the half of the cycle ($\omega t \approx 80^\circ$), the local minima begin to deviate from the direction of stretching and eventually attain the global minimum of $\pm 51^\circ$ during the remaining part of the cycle (Fig. 7). The reason for this is that initially the substrate strain u_x is small and thus Eq. (13) holds, which implies a parallel alignment. As stretching progresses, u_x increases and the fiber strain builds up to the level that Eq. (13) does not hold. Since the perpendicular alignment is not possible, the only stable configuration is an oblique alignment which corresponds to the fiber strain of $u_n = (1 + \alpha)/(\beta - 1)$ and where θ is determined by Eq. (12). Experimental data from the literature¹⁵ show, however, that during pure uniaxial stretching of cells in which contractile force generation is inhibited by blocking Rho activities, SFs, and FAs tend to align parallel with the direction of substrate stretching (i.e., $\theta \approx 0^\circ$), a result which is at odds with the oblique alignment of $\theta = \pm 51^\circ$. However, inhibition of Rho activities also inhibits SF polymerization and FA formation,¹⁵ which would imply that geometrical and material properties of SFs and FAs may also be altered. For example, by reducing A_{FA} from 3 to $1 \mu\text{m}^2$, h_{FA} from 100 to 50 nm, and the SF diameter from 300 to 200 nm, while keeping all other geometrical and material parameter values unaltered, we obtain $\alpha = 0.075$ and $\beta = 5.24$. With these values and $u_0 = 0.05$ and

$(u_x)_{\max} = 0.1$, the condition given by Eq. (13) is satisfied throughout the cycle and thus the model predicts the global minimum at $\theta = 0^\circ$. It is noteworthy, however, that the experimental data from Kaunas *et al.*¹⁵ indicate that when Rho activities are inhibited, not all SFs are aligned closely to the direction parallel with the direction of stretching; some of the SFs have notable oblique alignments. Thus, the simulations from Fig. 6 may at least qualitatively reflect the behavior of those SFs, suggesting that especially in the absence of Rho activity, the size of SFs and FAs is critical for their alignment at low pre-strains.

The model can also be used to predict SF–FA realignment in the case of non-uniform biaxial stretching by setting a value for the Poisson's ratio between -1 and 0 . We obtain for $\nu = -0.35$, $u_0 = 0.1$, and all other parameters the same as above, that the global minimum corresponds to $\theta = \pm 90^\circ$ (see Supplementary Material).

As a test of robustness of the model, we examine the sensitivity of the global minimum to variations in α and β . We consider the cases of the simple (i.e., $\nu = 0.35$) and the pure (i.e., $\nu = 0$) uniaxial stretching when $u_0 = 0.1$. We find that a 20% increase in both α and β causes a shift in the global minima toward on average $\sim 13\%$ higher angles during the simple uniaxial stretching, whereas it has no effect on the position of global minima during the pure uniaxial stretching. On the other hand, a 20% decrease in both α and β causes a shift in the global minima toward on average $\sim 31\%$ lower angles during the simple uniaxial stretching and 33% lower angles during the pure uniaxial stretching.

Discussion

In this study, we developed a mathematical model which describes realignment of an SF–FA assembly in response to substrate stretching as a mechanical stability phenomenon. Within this framework, it is revealed that mechanical stability should be considered as an important determinant of cytoskeletal rearrangement. We showed that geometrical and elastic properties of both SFs and FAs play a key role in determining stable alignments of the SF–FA assembly, with FAs being essential for directionality of those alignments. This is the novel and the most significant result of this study. This, however, does not preclude the contributions of Rho kinase activation,¹⁵ ATP-dependent processes, calcium influx,¹¹ and focal adhesion kinase activation^{26,34} to the cytoskeletal and FA rearrangements, since these processes facilitate and tune the cell's mechanosensing ability and influence remodeling of SFs and FAs. While realignment of SFs and FAs is a chemically mediated process, their final configuration must satisfy the minimum energy requirement and hence their realignment can be studied as a problem of stability. The consistency of the model predictions with the experimental data suggest that the extent of the non-mechanical contributions is rather limited for the purpose of the mechanisms in question, or that these contributions are already integrated in the analysis via their mechanical effects (i.e., pre-strain of the SFs; the stress-dependence of the chemical potential of SFs and FAs).

We next critically evaluate the model assumptions.

We assumed that SFs are linearly elastic (Eq. 2a) for the following reasons. First, experiments on isolated SFs showed that their stress–strain behavior can be regarded as linear for strains $< 40\%$.⁶ Since under normal physiological conditions cells usually do not experience strains that exceed 40% , stretched SFs would likely remain within the linear elasticity limit. Second, even if strains were sufficiently high to probe the non-linear region of the SF's stress–strain relationship, this non-linearity would not fundamentally alter predictions of the linearly elastic model. The reason is that experimental data show that the nonlinear stress–strain behavior of SFs is characterized by a convex strain-energy function.⁶ Only if the strain-energy function were non-convex, the model predictions would differ from the linear case.¹⁹

We assumed ad hoc that FAs are linearly elastic. While there is no direct evidence for elastic behavior of FAs, one may argue that the ability of FAs to rapidly remodel in response to mechanical stress may result in a non-linear elastic behavior. This rapid remodeling may also explain the wide variation in the reported FA stiffness. Based on the parameter values used to obtain simulations in Figs. 5–7, the SF strain ranges between 10^{-2} and 10^{-1} during a single stretching cycle. Taking this, the linear stress–strain relation for SFs, and Eq. (6) into account, we estimate the range of the average FA stress during a stretching, $\tau \sim 10^1$ – 10^2 kPa. Thus, for FAs to behave as a linearly elastic material, their stiffness E_{FA} should be close to the upper bound of the reported range of values. Higher values of E_{FA} would lower parameter β (see Eq. 8b) which, in turn, would favor SF alignment parallel with the stretching direction. We have also carried out simulations for the case of large, nonlinear SF strains, i.e., when $\varepsilon = u_n + u_n^2/2$, and obtained graphs that are qualitatively similar to those shown in Figs. 5–7 for the linear case (i.e., $\varepsilon = u_n$). Thus, for the sake of simplicity and mathematical transparency, we used the linear strain approximation.

In living cells, SFs exhibit a viscoelastic behavior,¹⁷ not an elastic one as we assumed in the model. Because of viscoelasticity as well as the dynamic nature of SF remodeling, FA formation and contractile force generation, realignment of SFs during substrate stretching should depend on the rate of stretching. Hsu *et al.*¹³ observed that during pure uniaxial cyclic stretching, SFs align perpendicularly when stretched at 1 Hz, while at 0.1 Hz cells show reduced SF alignment and no alignment at 0.01 Hz. To explain these observations, the authors proposed a dynamic model of SF alignment in which they included viscoelasticity of SFs as well as the rate of their assembly and disassembly. De *et al.*⁵ invoked cytoskeletal viscoelasticity to explain the directionality of the whole cell orientation during uniaxial cyclic stretching. Assuming that cells are characterized by a viscoelastic relaxation time constant (~ 1 s), their model predicts that if the stretching rate is faster than the cell's intrinsic time constant, then the cell would orient perpendicular to the direction of stretching, whereas if the stretching rate is slower than the time constant, the cell would orient parallel with the direction of stretching. Since our model is based on minimization of the potential energy, it cannot include viscoelasticity and remodeling of SFs or any other energy dissipative behaviors. While this is a limitation of the model, the good agreements between model predictions and experimental data from the literature obtained at 1 Hz stretching frequency^{13,15,28,33} suggest that the assumption of SF and FA elasticity may be appropriate for describing those observations.

We also assumed that $N_{SF}\mu_0^{SF}$ and $N_{FA}\mu_0^{FA}$ do not depend on the orientation of the SF–FA assembly. While this may be a reasonable assumption for μ_0^{SF} and μ_0^{FA} , it may not be for N_{SF} and N_{FA} . If the chemical potential of the aggregated molecules is lower than the chemical potential of free building molecules in the cytosol, then the nonassembled molecules would tend to join the aggregate and therefore SFs and FAs would polymerize.^{12,24} This process would be the greatest in the direction of substrate stretching where the chemical potential of SFs and FAs is the smallest due to the highest level of mechanical stress induced by stretching. Therefore, in reality, N_{SF} and N_{FA} may be a function of the initial orientation of the SF–FA assembly. In that regard, it is noteworthy that the observed tendency of SFs and FAs to orient away from the direction of stretching suggests that their alignment is not driven by polymerization, but rather by their tendency to attain mechanically stable configurations.

We considered individual SFs, whereas in living cells they are an integral part of the cytoskeletal network. Because molecular assembly events in the cell are influenced by mechanical stress which is distributed across the cytoskeletal network, it is likely that the network plays a role in the reassembly of SFs and FAs in response substrate stretching.²⁷ The CSK also contains a number of other polymer structures. For example, microtubules (MTs) often meet SFs at FAs¹⁶ and therefore the contractile stress of SFs is resisted not only by the

traction forces but also by compression of internal MTs.²⁷ This complementary force balance between contractile SFs, compression-bearing MTs and traction-bearing FAs influences their assembly.²⁷ On the other hand, the contribution of MTs to the energy budget and thus the global stability of the SF–FA–MT model seems to be minor, as we showed previously in our stability-based analysis of durotaxis.²⁰ Furthermore, experimental observations showed that CSK-based MTs do not undergo as drastic rearrangements in response to substrate stretching as SFs do.¹¹ Hence, in a first attempt, it seems reasonable to focus only on a single SF without the complications associated with network effects and/ or other cytoskeletal molecules.

While our model is reasonable for describing reorientation of SFs and FAs in two-dimensional cell cultures, where SFs are prominent and FAs are discrete, it may not be the case in three-dimensional cultures which often lack SFs and discrete FAs.^{9,10} Therefore the mechanisms that govern cell reorientation in three-dimensional cultures in response to external mechanical signaling may be quite different from those in two-dimensional cultures. While three-dimensional cultures represent a realistic environment for many cell types, in some cell types (e.g., vascular endothelial cells or pulmonary epithelial cells) their *in vivo* environment is effectively two-dimensional and thus, the model may be a good representation of SF reorientation in those cells *in vivo*.

In summary, our model shows that the geometrical and elastic properties of SFs and FAs are needed to predict SF alignments in response to uniaxial substrate stretching. While model predictions that are obtained for physiologically feasible parameter values are consistent with experimental data from the literature, further experiments in which the geometrical and the elastic properties of SFs and FAs will be measured are needed to support the model.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix

Derivation of Eq. (6)

We assume a one-dimensional state of stress in both the SF and FAs in the direction of the SF axis. We further assume, based on the one-dimensional model of FA by Shemesh *et al.*²⁴ that at the distal end (i.e., $\xi = 0$) the FA resist the traction T , and at the proximal end the FA resists the pulling stress σ of the SF. Assuming that the pulling stress from the SF and the traction from the substrate are transmitted to the FA linearly along its length L_{FA} , it follows that at a distance ξ from the distal end the stress transmitted from the SF to the FA is $\sigma_\xi = (\xi/L_{FA})\sigma$ and the traction transmitted from the substrate to the FA is $T_\xi = (1 - \xi/L_{FA})T$ (see Fig. 1). While both the traction and the SF stress are transmitted to the FA over L_{FA} , the corresponding contact SF–FA and FA–substrate areas are not the same. This is necessary to provide consistency of the force balance of the free-body diagram shown in Fig. 1.

Mechanical equilibrium demands that at every point ξ of the FA the stresses τ_ξ , σ_ξ , and T_ξ are balanced, i.e., that $\sigma_\xi = \tau_\xi + T_\xi$, from which we obtain that $\tau_\xi = \sigma_\xi - T_\xi = (\sigma + T)(\xi/L_{FA}) - T$. Using this expression, we obtain the average stress τ within the FA as follows

$$\tau = \frac{1}{L_{\text{FA}}} \int_0^{L_{\text{FA}}} \tau_{\xi} d\xi = 0.5 (\sigma - T). \quad (\text{A1})$$

Equilibrium of the whole SF–FA assembly requires that the tensile force due to σ and the force due to the traction are balanced, i.e., that

$$\sigma A_{\text{SF}} = \int_{A_{\text{FA}}} T_{\xi} dA = b \int_0^{L_{\text{FA}}} T_{\xi} d\xi, \quad (\text{A2})$$

where A_{SF} is the cross-sectional area of the SF, A_{FA} is the interfacial area of the FA, and b is a constant, representing the width of the FA at the interface with the substrate, i.e., $A_{\text{FA}} = bL_{\text{FA}}$. By substituting the expression for T_{ξ} into Eq. (A2), we obtain that

$$T = 2 \frac{A_{\text{SF}}}{A_{\text{FA}}} \sigma. \quad (\text{A3})$$

By substituting Eq. (A3) into Eq. (A1), we obtain Eq. (6).

Derivation of Eq. (10)

To obtain a relationship between u_n , u_0 , and u_x for simple uniaxial stretching, we follow the steps described in Lazopoulos and Pirentis.¹⁹ We assume that the SF is in the substrate xy -plane, at angle θ with respect to the x -axis, and that it carries an initial pre-strain u_0 . The substrate is stretched uniaxially in the direction of the x -axis, with the displacement gradient u_x (Fig. 2). Due to the Poisson's effect, the substrate also deforms in the y -direction with the displacement gradient $u_y = -\nu u_x$, where ν is the Poisson's ratio. The deformation gradient tensor $\mathbf{F}$ of the SF is a product of the deformation gradient of the substrate $\mathbf{F}_{\text{sub}}$ and the deformation gradient $\mathbf{F}_0$ of the SF due to the pre-strain, where xy -Cartesian components of $\mathbf{F}_{\text{sub}}$ and $\mathbf{F}_0$ are given as follows

$$\begin{aligned} \mathbf{F}_{\text{sub}} &= \begin{pmatrix} 1+u_x & 0 \\ 0 & 1-\nu u_x \end{pmatrix} \quad \text{and} \\ \mathbf{F}_0 &= \begin{pmatrix} \cos\theta & -\sin\theta \\ \sin\theta & \cos\theta \end{pmatrix} \begin{pmatrix} 1+u_0 & 0 \\ 0 & 1 \end{pmatrix} \\ &\quad \times \begin{pmatrix} \cos\theta & \sin\theta \\ -\sin\theta & \cos\theta \end{pmatrix}. \end{aligned} \quad (\text{A4})$$

From Eqs. (A4), we obtain $\mathbf{F} = \mathbf{F}_{\text{sub}} \mathbf{F}_0$. Using the right Cauchy-Green strain tensor $\mathbf{C} = \mathbf{F}^T \mathbf{F}$, where the superscript T indicates transpose of tensor, we calculate the displacement gradient (u_n) of the SF as follows $u_n = \sqrt{\mathbf{C} \mathbf{n} \cdot \mathbf{n}} - 1$, where $\mathbf{n} = (\cos \theta, \sin \theta)$ is a unit vector parallel with the SF (see Fig. 2), as follows

$$u_n = -1 + \frac{1+u_0}{\sqrt{2}} \sqrt{2+u_x[2(1-\nu)+u_x(1+\nu^2)]+u_x[2(1+\nu)+u_x(1-\nu^2)]\cos 2\theta}. \quad (\text{A5})$$

For small u_x , i.e., $u_x \ll 1$ and $u_x u_0 \ll 1$, Eq. (A5) can be linearized and we obtain u_n as given by Eq. (10).

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Abbreviations

SF	Stress fiber
FA	Focal adhesion
CSK	Cytoskeleton

Figure 1.

A schematic depiction of a stress fiber (SF), focal adhesion (FA), and substrate interaction (top) and the corresponding free-body diagram (bottom). h_{FA} is the thickness of the FA; σ is the stress within the SF; A_{SF} is the cross-sectional area of the SF; τ is the average shear stress within the FA; A_{FA} is the contact area between the FA and the substrate; T is traction at the distal end of the FA. The gray triangles indicate distributions of the SF stress and of the traction at the SF–FA and the substrate–FA interfaces, respectively. While the drawing suggests that the FA is under shear stress, the state of stress in both the FA and the SF is one-dimensional, along the ξ -axis.

Figure 2.

A stress fiber (SF)–focal adhesion (FA) assembly lies in the substrate xy -plane. The assembly is oriented at angle θ with respect to the x -axis which is parallel with the direction of substrate stretching. The vector $\mathbf{n}$ is the unit vector in the direction of the SF.

Figure 3.

The angle of orientation (θ) of the stress fiber-focal adhesion assembly for a wide range of values of parameters A_{FA}/A_{SF} and h_{FA}/L_{SF} and for $E_{SF}/E_{FA} = 150$, $u_0 = 0.1$, $u_x = 0.1$, and $\nu = 0.35$ for case (b) in the text when the total potential includes only the elastic potentials of the SF and FAs and the potential of the contractile stress σ . The gray scale shows the angle θ . Note that the only possible values are 0° or 90° . Calculations were carried out for a discrete number of points which, for better visualization, were sampled logarithmically.

Figure 4.

(a) The angle of orientation (θ) of the stress fiber-focal adhesion assembly for a wide range of values of parameters A_{FA}/A_{SF} and h_{FA}/L_{SF} and for $E_{SF}/E_{FA} = 150$, $u_0 = 0.1$, $u_x = 0.1$, and $v = 0.35$. The gray scale shows the angle θ for case (c) in the text when the total potential includes the elastic and the chemical potentials of the SF and FAs and the potential of the contractile stress σ . (b) Probability density of θ obtained from the data in (a). Calculations were carried out for a discrete number of points which, for better visualization, were sampled logarithmically (a), whereas for the probability density diagrams the data were sampled linearly (b).

Figure 5. Potential vs. stress fiber orientation (θ) relationships for simple uniaxial stretching during one cycle, for pre-strain $u_0 = 0.1$, amplitude $u_m = 0.05$, and substrate Poisson's ratio of $\nu = 0.35$. The potential is scaled with the volume of the stress fiber V_0^{SF} .

Figure 6. Potential vs. stress fiber orientation (θ) relationships for pure uniaxial stretching during one cycle for pre-strain $u_0 = 0.1$, amplitude $u_m = 0.05$. The potential is scaled with the volume of the stress fiber V_0^{SF} .

Figure 7. Potential vs. stress fiber orientation (θ) relationships for pure uniaxial stretching during one cycle for pre-strain $u_0 = 0.05$, amplitude $u_m = 0.05$. The potential is scaled with the volume of the stress fiber V_0^{SF} .