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A Mathematical Model of Cell Reorientation in Response to Substrate Stretching

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Abstract: It is well documented that in response to substrate stretching adhering cells alter their orientation. Generally, the cells reorient away from the direction of the maximum substrate strain, depending upon the magnitude of the substrate strain and the state of cell contractility. Theoretical models from the literature can describe only some aspects of this phenomenon. In the present study, we developed a more comprehensive mathematical model of cell reorientation than the current models. Using the framework of theory of non-linear elasticity, we found that the problem of cell reorientation was a stability problem, with the global (Maxwell's) criterion for stability. For the case of uniaxial substrate stretching, we showed that cells would orient away from the direction of substrate strain such that the angle between the cell long axis and the direction of the substrate strain would increase with increasing magnitude of the strain. We also showed that at a given substrate strain this angle would be greater in cells having greater contractile strain. These results are consistent with experimental observations reported in the literature.

keyword: Cell reorientation, Global stability, Contractility, Cytoskeleton, Substrate strain.

1 Introduction

Adherent cells change their orientation in response to nonuniform substrate stretching (Dartsch and Hämmerle, 1986; Iba and Sumpio, 1991; Kaunas et al., 2005; Neidlinger-Wilke et al., 2001; Sipkema et al., 2003; Takemasa et al., 1997; Wang et al., 2001; Wille et al., 2004). It has been shown that cell reorientation depends on the magnitude of applied substrate strain, on the magnitude of mechanical contractile stress of the cytoskeleton, and on Rho activity. The greater the magnitude of the substrate strain (Dartsch and Hämmerle, 1986; Kaunas et al., 2005; Neidlinger-Wilke et al., 2001; Takemasa et al., 1997) and of the contractile stress (Kaunas et al., 2005), the greater the departure of cell orientation from the direction of substrate stretching. Reduced contractility, on the other hand, leads to a closer alignment with the direction of stretching (Wang et al., 2001). In cells with normal Rho activitys stress fibers orient perpendicular to substrate stretching, while those with inhibited Rho activity, stress fibers align with the direction of substrate stretching (Kaunas et al., 2005).

To explain how cells change their orientation in response to mechanical signals from their microenvironment, Bischofs and co-workers (2003, 2004) developed a theoretical model based on the theory of linear elasticity that describes how an adherent cell may use its contractile apparatus in order to position and orient itself on the substrate. A key premise of their model is that the cell favors the orientation entailing position that requires the smallest mechanical work invested by the cell's contractile apparatus to build up a certain contractile force. This model predicts that a cell always aligns with the direction of substrate stretching, a result that is not consistent with a number of experimental circumstances where cells orient away from the stretching direction. Recently, Stamenović(2005) proposed a model of cell reorientation that leads to a simple mechanism – the contractile torque – that arises in response to nonuniform substrate stretching and steers the cell towards a new orientation. According to this model, the cell will orient perpendicularly to the direction of the greatest substrate strain if the contractile stress changes faster than linearly with the substrates strain, and with the direction of the greatest substrate strain if the contractile stress changes slower than linearly with the substrates strain. However, these predictions are independent on the magnitude of the substrate strain and on the magnitude of the contractile stress, whereas experimental data indicate otherwise.

In this study, we developed a mathematical model that could provide a more comprehensive description of cell orientation than the existing models. We studied this

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problem using a nonlinear elasticity and an energy minimization (i.e., stability) approach in order to determine cell orientations in response to uniaxial substrate stretching for different levels of the contractile stress. Model predictions were consistent with experimental data from the literature.

Model

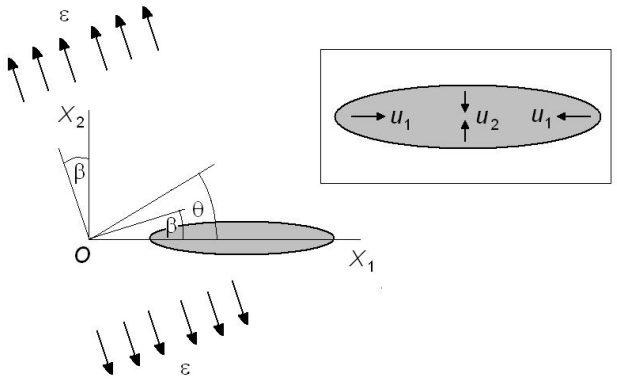

Figure 1 : A schematic representation of the cell-substrate system. Initially, the cell is oriented along the X_1-axis. Uniaxial substrate strain (ε) is applied to the cell at angle π/2 + β relative to the X_1 axis. As a result, the cell reorients by angle θ relative to the X_1-axis. *Inset:* the initial contractile displacement filed given by components u_1 and u_2 in the X_1- and X_2-directions, respectively.

We approached cell reorientation as a two-dimensional (2D) problem where a 2D cell adheres to a 2D substrate. The cell was initially aligned with the X_1-axis of a planar OX_1X_2 coordinate system (Fig. 1). To maintain mathematical transparency and computational simplicity, we introduced the following simplifying assumptions.

First, to mimic the effect of contractile stress, we assumed that at the reference state there was a pre-existing homogeneous displacement field in the cell, $\mathbf{u}(\mathbf{X}) = (u_1(X_1), u_2(X_2))$ caused by the contractile stress (Fig. 1, inset). A homogeneous displacement field implies that $\mathbf{u}(\mathbf{X})$ is a linear function of $\mathbf{X}$. Then, the deformation gradient $\mathbf{F}$ is independent of $\mathbf{X}$ and is given as

$$\mathbf{F} = \mathbf{I} + \nabla\mathbf{u} = \begin{pmatrix} 1+u_{1,1} & 0 \\ 0 & 1+u_{2,2} \end{pmatrix} \tag{1}$$

where $u_{i,j} = \partial u_i/\partial X_j$ (i,j = 1,2). From Eq. 1, we obtained the Cauchy-Green strain tensor $\mathbf{C}$ as

$$\mathbf{C} = \mathbf{F^T F} = \begin{pmatrix} (1+u_{1,1})^2 & 0 \\ 0 & (1+u_{2,2})^2 \end{pmatrix} \quad . \tag{2}$$

The principal invariants of $\mathbf{C}$, I_1 and I_2, were obtained from Eq. 2 as

$$I_1 = tr\mathbf{C} = (1+u_{1,1})^2 + (1+u_{2,2})^2 \tag{3}$$

$$I_2 = det\mathbf{C} = (1+u_{1,1})^2 (1+u_{2,2})^2 \tag{4}$$

Second, the cell was assumed to be non-linear elastic and isotropic, with elastic properties described by the Mooney-Rivlin strain-energy function

$$W = \frac{a_1}{2}(I_1 - 2) + \frac{a_2}{2}(I_2 - 2) \tag{5}$$

where a_1 and a_2 are constants.

Note that for the purpose of our model, mechanical properties of the substrate did not need to be specified (see Discussion).

Using Eqs. 3-5, we calculated the First Piola-Kirchhoff stress tensor, $P_{ij} = \partial W/\partial F_{ij}$ (i,j = 1,2)

$$P_{11} = 2(1+u_{1,1}) \times \left\{ \begin{array}{l} a_1\left(2u_{1,1} + u_{1,1}^2 + u_{2,2}^2 - 2u_{2,2}\right) + \\ a_2(u_{2,2}-1)^2\left(-1 + 2u_{1,1}(u_{2,2}-1)^2\right. \\ \left. + u_{1,1}^2(u_{2,2}-1)^2 - 2u_{2,2} + u_{2,2}^2\right) \end{array} \right\} \tag{6}$$

$$P_{22} = 2(1+u_{2,2}) \times \left\{ \begin{array}{l} a_1\left(2u_{2,2} + u_{2,2}^2 + u_{1,1}^2 - 2u_{1,1}\right) + \\ a_2(u_{1,1}-1)^2\left(-1 + 2u_{2,2}(u_{1,1}-1)^2\right. \\ \left. + u_{2,2}^2(u_{1,1}-1)^2 - 2u_{1,1} + u_{1,1}^2\right) \end{array} \right\} \tag{7}$$

$$P_{12} = P_{21} \equiv 0. \tag{8}$$

Since the displacement field $\mathbf{u}(\mathbf{X})$ was homogeneous, the corresponding stress field $\mathbf{P} = \mathbf{P}(\mathbf{X})$, given by Eqs. 6-8, identically satisfied the balance of linear momentum equation, $div\mathbf{P} = 0$, and the balance of angular momentum equation, $\mathbf{PF}^T = \mathbf{FP}^T$.

Equations 6-8 represent the contractile stress field $\mathbf{P} = \mathbf{P}(\mathbf{X})$ in the cell prior to application of an external load. This stress field is often termed "prestress" (cf. Wang et al., 2002). To distinguish this prestress and associated displacement field from those that were obtained after imposing substrate stretching, in the further text we labeled the former with the superscript '0'.

We next applied a uniaxial substrate stretch to the cell, characterized by strain ε andε in the direction π/2 + β relative to theX_1-axis (Fig. 1). We obtained the following displacement field after stretching

$$u_1 = u^0_{1,1}X_1 - \varepsilon(-X_1\sin\beta + X_2\cos\beta)\sin\beta$$
$$u_2 = u^0_{2,2}X_2 + \varepsilon(-X_1\sin\beta + X_2\cos\beta)\cos\beta. \quad (9)$$

The displacement field given by Eqs. 9 is homogeneous. From this displacement field, we obtained the deformation gradient **F**

$$\mathbf{F} = \begin{pmatrix} 1+u_{1,1} & u_{1,2} \\ u_{2,1} & 1+u_{2,2} \end{pmatrix} = \begin{pmatrix} 1+u^0_{1,1}+\varepsilon\sin^2\beta & -\varepsilon\sin\beta\cos\beta \\ -\varepsilon\sin\beta\cos\beta & 1+u^0_{2,2}+\varepsilon\cos^2\beta \end{pmatrix}. \quad (10)$$

Using the same procedure as above, we calculated **C** and **P** that corresponded to **F** (Eq. 10). Such obtained stress field **P** = **P**(**X**) identically satisfied the balance of linear momentum and the balance of angular momentum.

We next examined whether for a given ε there existed an orientation of the cell such that the total potential energy was minimum. Suppose there was such an orientation given by an angle θ relative to the X_1-axis (Fig. 1). We wanted to calculate θ since it would indicate a new equilibrium position of the cell after substrates stretching.

By rotating the cell through θ, we obtained from Eqs. 9 a new displacement field given as follows

$$u_1 = u^0_{1,1}X_1\cos\theta - u^0_{2,2}X_2\sin\theta - \varepsilon(-X_1\sin\beta + X_2\cos\beta)\sin\beta \quad (11)$$

$$u_2 = u^0_{1,1}X_1\sin\theta + u^0_{2,2}X_2\cos\theta + \varepsilon(-X_1\sin\beta + X_2\cos\beta)\cos\beta.$$

Equations 11 represent a homogeneous displacement field. From these equations, we calculated **F**

$$\mathbf{F} = \begin{pmatrix} 1+u^0_{1,1}\cos\theta+\varepsilon\sin^2\beta & -u^0_{2,2}\sin\theta-\varepsilon\sin\beta\cos\beta \\ u^0_{1,1}\sin\theta-\varepsilon\sin\beta\cos\beta & 1+u^0_{2,2}\cos\theta+\varepsilon\cos^2\beta \end{pmatrix} \quad (12)$$

and from Eq. 12 we obtained **C**, W and **P** as described above. To obtain stable values of θ, we proceed as follows.

The total potential energy density (V) was defined as

$$V \equiv W - P_{11}(F_{11}-1) - P_{12}F_{12} - P_{21}F_{21} - P_{22}(F_{22}-1) \quad (13)$$

where W was given by Eq. 5. Stable equilibrium values of θ have to satisfy $dV/d\theta = 0$ and $d^2V/d\theta^2 > 0$. We found that for small ε, the stable equilibrium configuration was θ = 0 (i.e., no reorientation). However, for larger values of ε, three non-trivial equilibrium values for θ were obtained. To determine which of these three values was stable, we used the global (Maxwell) criterion for stability (cf. Ericksen 1991), i.e., the cell would orient in the direction where its potential energy density attains a global minimum.

2 Results

We used the above model to predict changes in cell's orientation θ in response to changes in the magnitude ε and direction β of the substrate strain and to changes in the contractile strain $u^0_{1,1}$ and $u^0_{2,2}$. In all cases, we assumed *ad hoc* that the material coefficients in the Mooney-Rivlin strain energy function (Eq. 5) had values of a_1 = 100 and a_2 = –7 (units of energy/area). All calculations were done using the Mathematica software.

We first considered the cell with contractile strains of $u^0_{1,1} = 0.05$ and $u^0_{2,2} = 0.02$ subjected to the uniaxial substrate strain of ε = 0.1ε = 0.1 in the direction π/2 + β = $\pi/2 + \pi/6$ (i.e., $\beta = \pi/6$). The reason we assumed that $u^0_{1,1} > u^0_{2,2}$ was that experimental data show that contractile stress is greater in the direction of the cell's long axis than the in the transverse direction (Butler et al., 2002). We obtained one equilibrium solution, θ = 0.03rad, and it was stable. By increasing the substrate strain to ε = 0.2, leaving all the other parameters the same, we again obtained one stable solution, θ = 0.13rad. For ε = 0.4, we obtained three equilibrium solutions θ_1 = –0.205 rad, θ_2 = –0.372 rad, and θ_3 = 0.516 rad, but only θ_3 was a stable solution. We repeated these calculations for strains of ε = 0.6, and 0.8 and in each case obtained three equilibrium solutions with only one which was stable, θ_3 = 0.745 rad and θ_3 = 0.905 rad, respectively. We did similar calculations for the cases of β of π/4 and π/3 and obtained similar trends as in the case of β = π/6, i.e., that stable equilibrium values of θ increased with increasing substrate strain ε (Fig. 2). This is consistent with the experimental observations in cultured endothelial cells

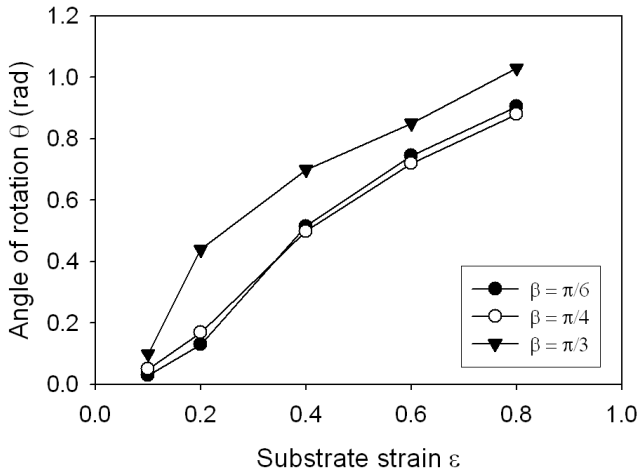

Figure 2 : The angle of cell reorientation increased (θ) with increasing substrate strain (ε) for different initial cell orientations given by angle β of π/6, π/4 and π/3 (for definitions of θ and β see Fig. 1). The predictions were obtained for contractile strains of $u^0_{1,1} = 0.05$ and $u^0_{2,2} = 0.02$.

Table 1 : Angle of cell orientation (θ) as a function of substrate strain (ε) for different values of contractile longitudinal ($u^0_{1,1}$) and transverse ($u^0_{2,2}$) strain. The values were obtained for $\beta = \pi/6$ (see Fig. 1).

ε	θ **(rad)**
$\boldsymbol{u^0_{1,1}}$ **= 0.05;** $\boldsymbol{u^0_{2,2}}$ **= 0.02**	
0.1	0.03
0.2	0.13
0.4	0.52
0.6	0.74
0.8	0.9
$\boldsymbol{u^0_{1,1}}$ **= 0.10;** $\boldsymbol{u^0_{2,2}}$ **= 0.02**	
0.1	0.1
0.2	0.35
0.4	0.65
0.6	0.82
0.8	0.98
$\boldsymbol{u^0_{1,1}}$ **= 0.20;** $\boldsymbol{u^0_{2,2}}$ **= 0.02**	
0.1	0.36
0.2	0.59
0.4	0.82
0.6	0.95
0.8	1.05

subjected to uniaxial substrate stretching (Dartsch and Hämmerle, 1986; Takemasa et al., 1997).

We next examined the effect of increasing cell contractility on cell reorientation. In all calculations, we gradually increased the longitudinal contractile strain $u^0_{1,1}$, while maintaining the transverse contractile strain constant, $u^0_{2,2} = 0.02$. We already indicated above that for ε = 0.1, $\beta = \pi/6$, and $u^0_{1,1} = 0.05$ and $u^0_{2,2} = 0.02$ the stable cell orientation was θ = 0.03 rad. We did the same calculations for $u^0_{1,1} = 0.1$ and $u^0_{2,2} = 0.02$ and for $u^0_{1,1} = 0.2$ and $u^0_{2,2} = 0.02$, keeping ε = 0.1 and $\beta = \pi/6$, and obtained one stable solution of θ = 0.10 rad and θ = 0.36 rad, respectively. We repeated the above procedure for ε of 0.4, 0.6 and 0.8 and for $\beta = \pi/6$. Similar trends were obtained (see Table 1). Taken together, these results indicated that the angle of rotation increased with increasing contractility. This is consistent with the experimental observations that the greater the contractile stress, the greater the rotation of the cell away from the direction of uniaxial substrate strain and vice versa (Kaunas et al., 2005; Wang et al., 2001).

3 Discussion

In this study, we developed a quantitative mathematical model that described how adherent cells changed their orientation as a function of uniaxial substrate stretching and of the contractile stress. The most favorable aspect of the model is that it could predict behaviors that were consistent with the experimental observations on living cells reported in the literature. In particular, the model predicted that a) an increase in the magnitude of substrate strain led to a systematic increase in the angle of cell orientation away from the direction of applied strain, and b) that this reorientation was enhanced by increasing contractile stress. To our knowledge, this is a first quantitative model that could describe these experimental observations. The model does not incorporate biochemical aspects of cell reorientation (e.g. the effect of Rho activities on cytoskeletal remodeling (Kaunas et al. (2005)). Rather, it offers a mathematical framework to determine mechanically stable configurations of the cell under a given substrate strain and a given contractile stress. Presumably the cell will favor a stable configuration to re-

orient and remodel its cytoskeleton accordingly.

Some comments should be made on the assumptions that were used in modeling and results that were obtained. The model used the framework of nonlinear theory of elasticity. The non-linearity included both kinematic and constitutive equations. The former is critical since large rotations could not be described using the formalism of linear elasticity. On the other hand, the assumed non-linear constitutive behavior, the Money-Rivlin equation (Eq. 5), was *ad hoc*. We could also use other types of constitutive equations in our model, including the neo-Hookean and the Hooke's law. This would most likely influence model predictions. Moreover, changes in values of parameters a_1 and a_2 in the Money-Rivlin equation also influence model predictions. For example, by altering values of parameters a_1 and a_2 it was possible to obtain, at a given substrate strain, several co-existing stable values of orientation angle θ (cf. Lazopoulos, 2005a,b). This, however, is consistent with experimental observations that different cell orientations may co-exist at a given substrate strain (Takemasa et al., 1997).

In the model, we assumed that the contractile strain field was homogeneous. This is an oversimplification of the realistic displacement field within living cells; it has been shown that the contractile displacement filed within living airway smooth muscle cells is not smooth but rather characterized by an inhomogeneous spatial distribution (Tolić-Nørrelykke et al., 2002). It is possible to include a non-homogeneous strain distribution in the model, but at the expense of simplicity and mathematical transparency of our approach. Thus, for demonstrating that cell reorientation could be treated as a stability problem, we kept our model very simple.

The range of the substrate strain ε in the model was 10 to 80%, within the range of experimental values (0-100%) reported by Takemasa et al. (1997). However, in many experimental studies, ε ranged between 10% and 20% (cf. Dartsch and Hämmerle, 1986; Iba and Sumpio, 1991; Neidlinger-Wilke et al., 2001; Sipkema et al., 2003; Wang et al., 2001; Wille et al., 2004; Kaunas et al., 2005). According to our model, for ε of 10-20%, the contractile stress has to be high in order for the cell to orient perpendicularly to the direction of substrate stretching (Table 1).

In the model, mechanical properties of the substrate were not needed to be specified; the substrate was viewed only as a medium for transmission of applied uniaxial strain to the cell. This is different from previous models where substrate stiffness played a key role in determining cell orientation (Bischofs et al., 2003, 2004). However, considering that those models could not predict that cells orient away from the direction of the applied strain, substrate stiffness may not be critical for cell reorientation. Nevertheless, if needed we can include deformability of the substrate in our model. Other simplifications included the assumption that the cell is an elastic material, whereas in reality it is a viscoelastic material. Moreover, we assumed that the cell-substrate is a 2D system whereas in reality the extracellular matrix it is a 3D system, which also may influence cell orientation. However, most experiments that have studied cell orientation have been conducted on 2D cultures and therefore our assumption seems reasonable.

In summary, we developed a mathematical model that is based on the idea that cell reorientation during substrate stretching is determined by the configuration where the cell attains a globally stable mechanical equilibrium. The model yielded quantitative predictions that were consistent with experimental observations on living cells, and which could not be obtained by previous models. Despite all the simplifications mentioned above, we believe, based on the good agreement between predictions and experimental data, that the model provides a good framework for quantifying cell reorientation in response to substrate stretching.

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