 MCB, vol.2, no.2, pp.69-76, 2005

Contractile Torque as a Steering Mechanism for Orientation of Adherent Cells

Dimitrije Stamenović [1]

Abstract: It is well established that adherent cells change their orientation in response to non-uniform substrate stretching. Most observations indicate that cells orient away from the direction of the maximal substrate strain, whereas in some cases cells also align with the direction of the maximal strain. Previous studies suggest that orientation and steering of the cell may be closely tied to cytoskeletal contractile stress but they could not explain the mechanisms that direct cell reorientation. This led us to develop a simple, mechanistic theoretical model that could predict a direction of cell orientation in response to mechanical nonuniformities of the substrate. The model leads to a simple physical mechanism – namely the contractile torque – that directs the cell toward a new orientation in response to anisotropic substrate stretching or substrate material anisotropy. A direction of the torque is determined by a dependence of the contractile stress on substrate strain. Model predictions are tested in the case of simple elongation of the substrate and found to be consistent with experimental data from the literature.

keyword: contractile dipole, mechanosensing, traction, contractile stress, strain, substrate.

1 Introduction

It is well documented in a variety of adherent cell types that cell orientation changes in response to nonuniform substrate stretching (Dartsch and Hämmerle,1986; Eastwood et al., 1998; Iba and Sumpio,1991; Ignatius et al. 2004; Neidlinger-Wilke et al., 2001; Sipkema et al., 2003; Takemasa et al., 1997; Wang et al., 2001; Wille et al., 2004). Much is known about the effect of stretch on cells, especially with regard to induced changes in gene expression. However, the upstream mechanisms, especially those by which mechanical stretching drives the cell to a new orientation, are largely unknown. Generally, cells tend to orient transverse to the direction of the maximal substrate strain (Dartsch and Hämmerle,1986; Iba and Sumpio,1991; Neidlinger-Wilke et al., 2001; Sipkema et al., 2003; Takemasa et al., 1997; Wang et al., 2001; Wille et al., 2004), but some observations have indicated that cells sometimes align with the direction of the maximal strain (Eastwood et al., 1998; Ignatius et al. 2004). It was also found that cell orientation is related to the magnitude of applied strain and on the state of cell contractility; the greater the magnitude of the applied strain, the greater the departure of cell orientation from the direction of strain (Neidlinger-Wilke et al., 2001; Takemasa et al., 1997) whereas reduced contractility leads to closer alignment with the direction of applied strain (Wang et al., 2001). Cells that are cultured in three-dimensional collagen gels tend to align in the direction of greatest strain (Bates and Martin, 1990; Eastwood et al., 1998). It is evident from these published reports that we do not understand why some cells in some conditions align away from the direction of external strain whereas some cells align with the direction of strain. Importantly, underlying mechanisms of cell reorientation in response to external mechanical perturbation are largely unknown.

Bischofs and co-workers (2003, 2004) have proposed a theoretical model to describe how an adherent cell may use its contractile apparatus in order to orient itself on the substrate. A key premise of their model is that the cell favors the orientation entailing the smallest mechanical work invested by the cell's contractile apparatus to build up a certain contractile force. However, it is not clear from their model how the cell sense what position leads to a minimal work. An implicit assumption is that the cell probes its physical environment randomly until it senses the optimal position and then aligns accordingly. However, there is no experimental evidence to back up this assumption. For example, their model predicts that a cell aligns with the direction of substrate stretching, but in many experimental circumstances observations contradict this prediction (Dartsch and Hämmerle,1986; Iba and Sumpio,1991; Neidlinger-Wilke et al., 2001; Sipkema et al., 2003; Takemasa et al., 1997; Wang et al.,

[1] Department of Biomedical Engineering, Boston University, Boston, Massachusetts

2001; Wille et al., 2004). Therefore, a new conceptual model and physical principles are needed to resolve this apparent contradiction and to elucidate mechanisms.

In this study, we proposed a theoretical model that could explain how cells might change their orientation in response to substrate mechanical nonuniformities, including nonuniform stretching and material anisotropy. A key mechanism embodied in the model that steers the cell toward a new orientation is a contractile torque that arises at the cell-substrate interface as a result of altered balance of contractile forces within the cell in response to mechanical disturbances from the substrate. Model predictions are compared with experimental data from the literature.

2 Model

When the cell adheres to its substrate via integrin-extracelluar matrix ligation, interfacial stresses (traction) arise as a result of endogenous contractile stresses generated via myosin-actin interactions. It has been shown that cells use this contractile stresses to probe rigidity of their substrate by "pinching" the substrate and then use this information to regulate their adhesion, magnitude and directionality of locomotion, growth and apoptosis (Lo et al., 2000; Pelham and Wang, 1997; Wang et al., 2000). The simplest metric of this cell pinching is known as the contractile dipole (Butler et al., 2002; Wang et al., 2002). It is a tensorial quantity whose magnitude, the contractile moment (M), can be computed directly from the spatial, two-dimensional distribution of tractions; M is a scalar quantity equivalent to a pair of imaginary point forces of equal magnitude (F) and opposite sense, separated by a distance (d) (i.e., dipole), $M = d \cdot F$ (Fig. 1).

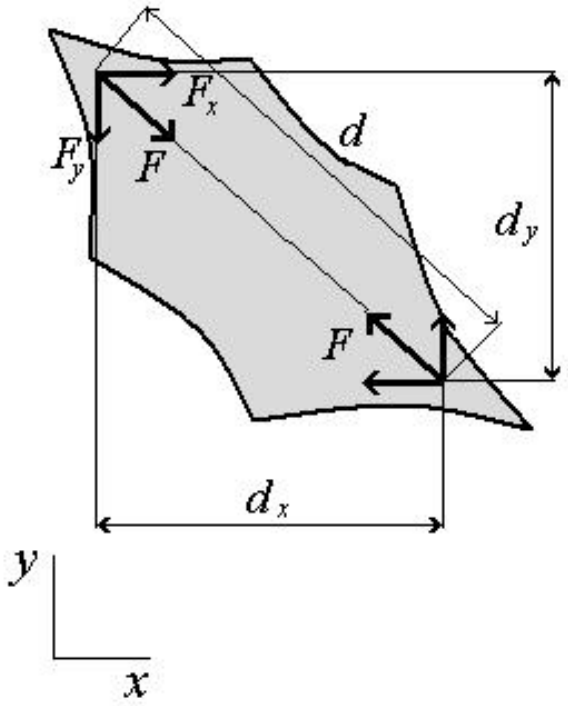

Figure 1 : Traction field that arises at the cell-substrate interface (gray area) in response to cell contraction can be replaced by a pair of point forces (F) separated by a distance (d). Together, they form a dipole whose strength, the contractile moment (M) equals the product $M = d \cdot F$. The x and y components of the contractile dipole; F_x and F_y are the x and y components of F and d_x and d_y are the corresponding components of d.

Mathematically, the contractile dipole tensor $\mathbf{M}$ is given as follows [Betler, et al.(2002)]

$$\mathbf{M} = \begin{pmatrix} d_x F_x & d_x F_y \\ d_y F_x & d_y F_y \end{pmatrix} = \begin{pmatrix} \rightarrow\leftarrow & \downarrow\uparrow \\ \uparrow\downarrow & \downarrow \\ & \uparrow \end{pmatrix} \qquad (1)$$

where F_x and F_y are the x and y (in plane) components of the contractile dipole force vector $\mathbf{F}$ and d_x and d_y are the corresponding components of the length d of the dipole (Fig. 1). (Here and in the further text the interfacial surface between the cell and the substrate is assumed to be in the xy-plane.) The arrows on the right-hand side of Eq. (1) are the schematic representation of the mechanical actions of $\mathbf{M}$: the diagonal terms $d_x F_x$ and $d_y F_y$ represent contractions in the $x-$ and y-directions, respectively; the off-diagonal terms $d_x F_y$ and $d_y F_x$ represent contractile torques in the counterclockwise and clockwise directions, respectively. Since the net torque produced by the dipole is zero, then the net torque produced by the x and y components of the dipole is also zero, i.e., $d_x F_y - d_y F_x = 0$. However, under mechanical disturbances of the substrate, a finite net torque may be generated. We hypothesize that it is this torque that directs the cell toward a new orientation. Two cases are considered: a) nonuniform (anisotropic) stretching of the substrate and b) material anisotropy of the substrate.

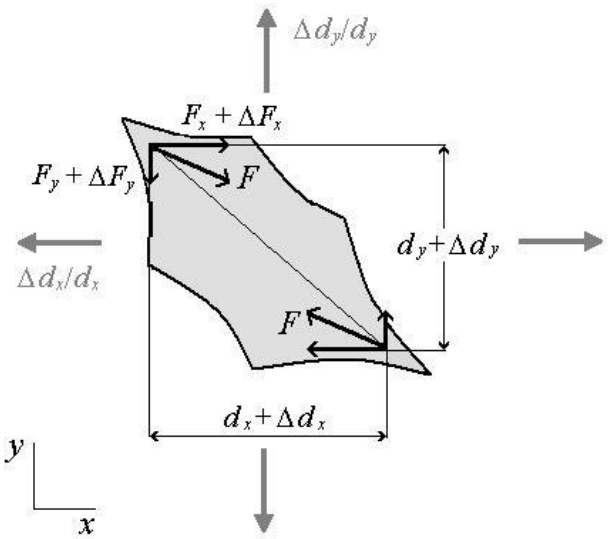

Figure 2 : In response to a biaxial stretch along $x-$ and y-axes (thick gray arrows), x and y components of the contractile dipole from Fig. 1 change; d_x and d_y change by Δd_x and Δd_y, and F_x and F_y change by ΔF_x and ΔF_y, respectively. The corresponding substrate x and y strains equal $\Delta d_x/d_x$ and $\Delta d_y/d_y$, respectively.

2.1 Nonuniform stretching

Suppose that the substrate is stretched biaxially, in the $x-$ and $y-$ directions, such that the strain in the x-direction is greater than in the y-direction. Then the x component of the dipole length, d_x, will increase by Δd_x and the y component, d_y, will increase by Δd_y such that $\Delta d_x/d_x > \Delta d_y/d_y$ (Fig. 2). The corresponding changes in F_x and F_y are ΔF_x and ΔF_y, respectively (Fig. 2). Then the net contractile torque (T) equals

$$T = (d_x+\Delta d_x)(F_y+\Delta F_y) - (d_y+\Delta d_y)(F_x+\Delta F_x) \qquad (2)$$

Taking into account that prior to stretching $d_xF_y - d_yF_x = 0$, it follows from Eq. (2) that

$$T = F_y d_x \left[\left(\frac{\Delta d_x}{d_x} - \frac{\Delta d_y}{d_y} \right) + \left(\frac{\Delta F_y}{F_y} - \frac{\Delta F_x}{F_x} \right) + \left(\frac{\Delta d_x}{d_x}\frac{\Delta F_y}{F_y} - \frac{\Delta d_y}{d_y}\frac{\Delta F_x}{F_x} \right) \right] \qquad (3)$$

The sign of the expression on the right-hand side of Eq. (3) determines the direction of cell reorientation; $T > 0$ indicates orientation toward the x-direction, i.e., the direction of the larger strain, whereas $T < 0$ indicates orientation away from the direction of the larger strain. It is clear from Eq. (3) that for $\Delta d_x/d_x > \Delta d_y/d_y$ only the second and the third terms may have negative contributions to T and that these contributions depend on if and how F_x and F_y change during stretching. To illustrate this, while maintaining mathematical simplicity and transparency, we assume thatF_x and F_y change proportionally to d_x^β and d_y^β, respectively, where β is a constant. The rationale for this assumption is that the a given value of β determines a type of dependence of F_x and F_y on d_x and d_y; β greater, less or equal to unity implies that F_x and F_y change faster than linearly, slower than linearly or proportionally to the applied substrate x and y strains, respectively. Taking into account this assumption, Eq. (3) becomes

$$T = F_y d_x \left[\left(\frac{\Delta d_x}{d_x} - \frac{\Delta d_y}{d_y} \right) (1-\beta) \right] \qquad (4)$$

If $\beta > 1$, then it follows from Eq. (4) that $T < 0$ and the cell would steer away from the x-direction (Fig. 3A), whereas if $\beta < 1$, then $T > 0$ and the cell would steer toward the x-direction (Fig. 3B). If $\beta = 1$, then $T = 0$ and the cell would not change its orientation (Fig. 3C).

The following special cases also are noteworthy. One is equibiaxial (uniform) stretching when $\Delta d_x/d_x = \Delta d_y/d_y$ and $\Delta F_x/F_x = \Delta F_y/F_y$. Then, it follows from Eq. (3) that $T = 0$ and therefore that cell would not alter its orientation, independent of particular relationships between F_x and F_y and the substrate strains (Fig. 3D). The second one is substrate compression. For simplicity, consider the case of pure uniaxial compression in the x-direction (i.e., $\Delta d_x/d_x < 0; \Delta d_y/d_y = 0$). Then Eq. (4) becomes

$$T = -F_y d_x \left| \frac{\Delta d_x}{d_x} \right| (1-\beta) \qquad (5)$$

In this case, $\beta > 1$ implies $T > 0$ (Fig. 3E) and $\beta < 1$, $T < 0$ (Fig. 3F), which is opposite from the directions that would be predicted in the case of pure uniaxial substrate stretching in the x-direction (i.e., $\Delta d_x/d_x > 0$; $\Delta d_y/d_y = 0$).

According to the model (Eqs. 3-5), the cell will stop changing its orientation once it attains a position where

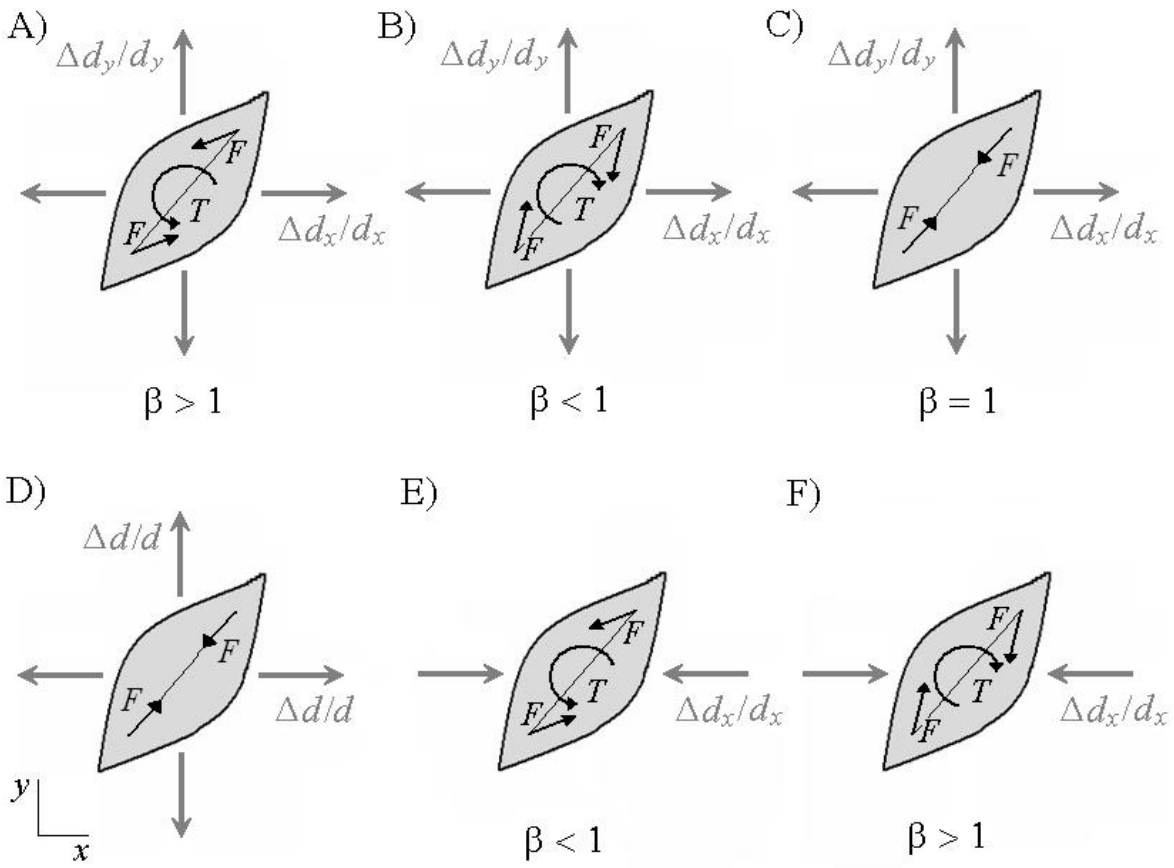

Figure 3 : A schematic representation of the direction of cell orientation during biaxial stretching based on predictions of Eq. (4). Here F denotes the contractile force and T the contractile torque, $\Delta d_x/d_x > \Delta d_y/d_y$ are substrate strains in the x- and y-directions, respectively. A) $T < 0$ and the cell steers away from the x-direction when the x and y components of F change faster than linearly with the corresponding substrate strains ($\beta > 1$). B) $T > 0$ and the cell steers in the x-direction when the x and y components of F change slower than linearly with the corresponding substrate strains ($\beta < 1$). C) $T = 0$ and the cell does not change its orientation when the x and y components of F change directly proportionally with the corresponding substrate strains ($\beta = 1$). D) During equibiaxial (uniform) stretching ($\Delta d_x/d_x = \Delta d_y/d_y \equiv \Delta d/d$), $T = 0$ and the cell does not change its orientation. During pure uniaxial compression in the x-direction ($\Delta d_x/d_x < 0, \Delta d_y/d_y = 0$) (Eq. 5), E) $T < 0$ and the cell steers away from the x-direction when the x and y components of F change slower than linearly with the corresponding substrate strains ($\beta < 1$), and F) $T > 0$ and the cell steers toward the x-direction when the x and y components of F change faster than linearly with the corresponding substrate strains ($\beta > 1$).

$T = 0$. This position is either the orientation with the x-direction (i.e., $F_y = 0$) or with the direction transverse to it, the y-direction (i.e., $d_x = 0$). No other orientations are possible.

To compare model predictions with experimental data from the literature, we considered a special case of substrate stretching – simple elongation – that has been used previously in experimental studies of cell reorientation (cf. Takemasa et al., 1997; Wang et al., 2001; Wille et al., 2004). During simple elongation in the x-direction the substrate shrinks in the y-direction, i.e., $\Delta d_y/d_y = -\nu \Delta d_x/d_x$, where $0 \leq \nu \leq 0.5$ is Poisson's Ratio of the substrate, while the contractile stress increases in the x-direction and does not change in the y-direction, i.e., $\Delta F_x/F_x > 0$ and $\Delta F_y/F_y = 0$ Thus, Eq. (3) becomes

$$T = F_y d_x \left[(1+\nu)\frac{\Delta d_x}{d_x} - \left(1 - \nu\frac{\Delta d_x}{d_x}\right)\frac{\Delta F_x}{F_x} \right] \quad (6)$$

Since stretchable substrates are usually made of elastomeric membranes that change little their volumes during cell stretching, it follows that $\nu \approx 0.5$. Taking this value into account, we predict from Eq. (6) values of $\Delta F_x/F_x$ for which $T > 0$ and $T < 0$, for substrate strains $\Delta d_x/d_x$ ranging from 0-14% (Fig. 4). These results are then compared with published experimental data for changes of contractile stress in response to substrate stretching obtained from living airway smooth muscle cells (Rosenblatt et al., 2004) and endothelial cells (Pourati et al., 1998). It is found that these experimental values fall in the range where the model predicts that $T < 0$, suggesting that the cells would orient away from the direction of stretching (Fig. 4). This finding is consistent with observed changes in orientation of living smooth muscle and endothelial cells during simple elongation of the substrate reported previously (cf. Dartsch and Hämmerle, 1986; Takemasa et al., 1997; Wang et al., 2001).

Model predictions for equbiaxial stretching are also compared with experimental data from the literature. The model predicts that during equibiaxial substrate stretching cells do not develop contractile torque and therefore they will not reorient. This prediction is consistent with previous observations that living endothelial cells do not exhibit preferential orientation during equibiaxial substrate stretching (Wang et al., 2001).

2.2 *Material anisotropy*

Our model can be applied to the case of material anisotropy of the substrate. Suppose that the substrate is stiffer in the $x-$ than in the y-direction. Then in response to cell contraction, the fractional decrease of d_x will be smaller than of d_y, i.e., $|\Delta d_x/d_x| < |\Delta d_y/d_y|$. For simplicity, let assume that the contractile force remains constant during contraction (isotonic contraction). In that case the net torque $T = F_y d_x(|\Delta d_y/d_y| - |\Delta d_x/d_x|) > 0$ and the cell will tend to align with the x-axis, i.e., the direction of the greater stiffness. The same result was obtained by Bischofs et al. (2003, 2004). Note that in the case of an isotropic substrate, $|\Delta d_x/d_x| = |\Delta d_y/d_y|$ and hence $T \equiv 0$ and the cell will not change its orientation during contraction. We could also consider the possibility that during cell contraction F_x and F_y change as well, and thus would obtain an expression for T similar to the one given by Eq. (3). However, there are no experimental data in the literature about the effect of substrate material anisotropy on cell orientation that can be compared with the model predictions. Thus, we do not consider the effect of material anisotropy in more detail.

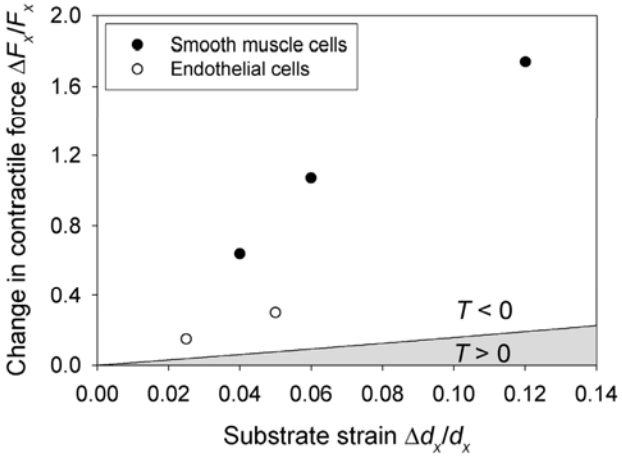

Figure 4 : Fractional change in contractile force ($\Delta F_x/F_x$) vs. substrate strain ($\Delta d_x/d_x$) during simple elongation of the substrate in the x-direction. The gray area indicates the region where the contractile torque is positive ($T > 0$) and the white region where it is negative ($T < 0$), as predicted by Eq. (6). Dots are experimental data from the literature for cultured airway smooth muscle cells (Rosenblatt et al., 2004) and endothelial cells (Pourati et al., 1998). The data lay in the region where $T < 0$ suggesting that those cells will orient away from the stretching direction.

3 Discussion

During the past decade, a number of studies have shown that contractile stress borne by the cytoskeleton plays a central role in mechanobiology of adherent cells. It confers shape stability to the cell which is important for adhesion, growth, locomotion, mechanosensing and apoptosis (cf. Bischofs and Schwarz, 2003; Ingber, 2003; Stamenović, 2005; Wang et al., 2000). It determines the rheological behavior of the cell via cytoskeletal remodeling (Bursac et al., 2005). It may also regulate nuclear functions through mechanical distension that it exerts on the nucleus (Hu et al., 2005). In this paper, we studied the

influence of cytoskeletal contractile stress on cell orientation. We developed a model which showed how cells might use this stress to sense mechanical signals from their microenvironment and reorient themselves accordingly. The most significant aspect of the proposed model is that it reveals a simple physical mechanism – namely the contractile torque – that directs the cell toward a new orientation in response to mechanical nonuniformities of the substrate. The model predicts that in the case of nonuniform, biaxial stretching the cell would align either with or transverse to the direction of the greater substrate strain, or would not change its initial orientation, depending on how contractile force changes during stretching. This is, to our knowledge, a first mechanistic explanation of why adherent cells sometimes orient away from the direction of substrate stretching and other times with the direction of substrate stretching. The model also predicts that on an anisotropic substrate the cell will align with the direction of greatest stiffness.

It is important to clarify that we do not consider the contractile torque as sole mechanism that propels the cell to a new orientation; i.e., it is not analogous to the dynamic torque like, for example, the one that causes a propeller of an airplane to rotate. Other, non-mechanical mechanisms are also likely to play important roles in cell reorientation. For example, it is well documented that during cell reorientation remodelings of the cytoskeleton and focal adhesions take place (Nicolas et al., 2001; Sipkema et al., 2003; Takemasa et al., 1997; Wang et al., 2004; Wille et al., 2004). It is also known that stretch-activated ion channels play important role in mechanosensing and that they may influence stretch-induced cell reorientation (Hayakawa et al., 2001). However, for all these non-mechanical events to occur, mechanical signaling from the cell microenvironment is required which, in conjunction with cytoskeletal contractile stress, generate the contractile torque that directs the cell toward a new orientation, as described in our model. Thus, the contractile torque may be viewed as a mechanism that initiates and directs a cascade of biochemical events that together drive the cell toward a new orientation. Since the contractile torque is directly measurable using the existing techniques (Bulter et al., 2002; Wang et al., 2002), it should not be difficult to experimentally verify whether this torque is closely associated with cytoskelatal and focal adhesion remodelings.

In the model, a nonlinear dependence of the contractile force on the substrate strain is critical for determining the direction of cell reorientation. This nonlinear behavior is likely to come from biopolymers of the cytoskeleton, including actin stress fibers (Sato et al., 2004) and intermediate filaments (vimentin) (Janmey et al., 1991; Wang and Stamenović, 2000) which have been shown to exhibit a marked, faster than linear dependence on tensile stress.

The model predicts that at the end of reorientation the cell would align either with or transverse to the direction of the maximal substrate strain, whereas experimental data show that cells may orient at some acute angle, between 0° and 90°, with respect to the maximal strain direction, depending on the state of cell contractility, magnitude of the substrate strain and other factors (cf. Neidlinger-Wilke et al., 2001; Takemasa et al., 1997; Wille et al., 2004). This is a limitation of the model and it possibly reflects model assumptions including the following: that contractile forces are elastic, whereas in reality they are non-elastic; that there is only one contractile dipole whereas in reality there are two principal dipoles (i.e., quadrupole) that correspond to the eigenvalues and the eigenvectors of the contractile matrix $\mathbf{M}$ (Eq. 1) (see Bischofs and Schwarz, 2003, Bischofs et al., 2004, Butler et al., 2002); that the cell is a two-dimensional system, whereas it is a three-dimensional system. Introducing all these factors into the model would most likely influence the model prediction and make mathematical relationships more complex and less transparent than in the present model. Nevertheless, the basic concept of the contractile torque as a steering mechanism of cell reorientation, as described in this study, would remain the same.

The experimental data that we used to compare with the model predictions for cell orientation during simple elongation of the substrate (Fig. 4) were not obtained from the direct measurements of contractile force during substrate stretching. Instead, we used data for changes in cell stiffness measured during uniform cell stretching. However, we showed previously that the cell stiffness increases in direct proportion with the contractile moment (Wang et al., 2002), and therefore changes in the cell stiffness may be used as an index of corresponding changes in the contractile force.

What can we learn by knowing what dictates orientation of adherent cells? Various studies have shown that orientation of adherent cells is important for physiological

functions of organs and tissues. For example, the orientation of the smooth muscle in the airway is very important in determining airway responsiveness (Bates and Martin, 1990). Importantly, this orientation in the airway might be altered in abnormal conditions such as asthma or inflammation in which the mechanical stretches/stresses might be altered through alterations of the mechanical properties of the extracellular matrix. This could further alter the effect of airway smooth muscle cell contraction on the degree of airway lumen narrowing.

4 Summary

The model proposed in this study reveals a simple physical mechanism, the contractile torque, that directs the cell toward a new orientation in response to mechanical nonuniformities of the substrate. It shows that the direction of the torque, and thereby the direction of cell orientation, is determined by a dependence of contractile stress on the applied substrate strain. The model predictions obtained for a simple elongation of the substrate are found to be consistent with experimental data from the literature.

Acknowledgement: This study was supported by NIH Grant HL-33009.

References

Bates, J. H.; Martin, J. G. (1990): A theoretical study of the effect of airway smooth muscle orientation on bronchoconstriction. *J Appl Physiol*, vol. 69, pp. 995-1001.

Bischofs, I. B.; Safran S.A.; Schwarz, U. S. (2004): Elastic interactions of active cell cells with soft materials. *Phys Rev E*, vol. 69, pp. 021911.

Bischofs, I. B.; Schwarz, U. S. (2003): Cell organization in soft media due to active mechanosensing *Proc Natl Acad Sci USA*, vol. 100, pp. 9274-9279.

Bursac, P.; Lenormad, G.; Fabry, B.; Oliver, M.; Weitz, D. A.; Viasnoff, V.; Butler, J. P.; Fredberg, J. J. (2005): Mechanism unifying cytoskeletal remodeling and slow dynamics in living cells. *Nature Materials*, vol. 4, pp. 557-561.

Butler, J. P.; Tolić-Nørrelykke, I. M.; Fabry, B.; Fredberg, J. J. (2002): Traction fields, moments, and strain energy that cells exert on their surroundings. *Am J Physiol Cell Physiol*, vol. 282, pp. C595-C605.

Dartsch, P. C.; Hämmerle, H. (1986): Orientation response of arterial smooth muscle cells to mechanical stimulation. *Eur J Cell Biol*, vol. 41, pp. 339–346.

Eastwood, M.; Mudera,V. C.; McGrouther, D. A.; Brown, R. A. (1998): Effect of precise mechanical loading on fibroblast populated collagen lattices: Morphological changes. *Cell Motil Cytoskeleton*, vol. 40, pp. 13-21.

Hayakawa, K.; Sato, N.; Obinata, T. (2001): Dynamic reorientation of cultured cells and stress fibers under mechanical stress from periodic stretching. *Exp Cell Res*, vol. 268, pp.104-114.

Hu, S.; Chen, J.; Butler, J. P.; Wang, N. (2005): Prestress mediates force propagation to the nucleus. *Biochem Biophys Res Commun*, vol. 329, pp. 432-428.

Iba, T.; Sumpio, B. E. (1991): Morphological response of human endothelial cells subjected to cyclic strain *in vitro. Microvasc Res*, vol. 42, pp. 245-254.

Ignatius, A.; Blessing, H.; Liedert, A.; Kaspar, D.; Kreja, L.; Friemert, B.; Claes L. (2004): Effects of mechanical strain on human osteoblastic precursor cells in type I collagen matrices. *Orthopade*, vol. 33, pp.1386-1393.

Ingber, D. E. (2003): Cellular tensegrity revisited I. Cell structure and hierarchical systems biology. *J Cell Sci*, vol. 116, pp. 1157-1173.

Janmey, P. A.; Euteneuer, U.; Traub, P.; Schliwa, M. (1991): Viscoelastic properties of vimentin compared with other filamentous biopolymer networks. *J Cell Biol*, vol. 113, pp. 155-160.

Lo, C.-M.; Wang, H.-B.; Dembo, M.; Wang, Y.-L. (2000): Cell movement is guided by the rigidity of the substrate. *Biophys J*, vol. 79, pp. 144-152.

Neidlinger-Wilke, C.; Grood, E. S.; Wang, J. H.-C.; Brand, R. A.; Claes, L. (2001): Cell alignment is induced by cyclic changes in cell length: studies of cells grown in cyclically stretched substrates. *J Orthop Res*, vol. 19, pp. 286-293.

Nicolas, A.; Geiger, B.; Safran, S. A. (2004): Cell mechanosensitivity controls the anisotropy of focal adhesions. *Proc Natl Acad Sci USA*, vol.101, pp.12520-12525.

Pelham RJ Jr; Wang Y-L. (1997) Cell locomotion and focal adhesions are regulated by substrate flexibility. *Proc Natl Acad Sci USA* vol.94, pp.13661-13665.

Pourati, J.; Maniotis, A.; Spiegel, D.; Schaffer, J. L.; Butler, J. P.; Fredberg, J. J.; Ingber, D. E.; Stamenović, D.; Wang, N. (1998): Is cytoskeletal tension a major determinant of cell deformability in adherent endothelial cells *Am J Physiol Cell Physiol* vol.274, pp.C1283-C1289.

Rosenblatt,N.; Hu, S.; Chen,J.; Wang, N.; Stamenović, D. (2004): Distending stress of the cytoskeleton is a key determinant of cell rheological behavior. *Biochem Biophys Res Commun*, vol. 321, pp. 617-622.

Sato, M.; Deguchi, S.; Ohashi, T. (2004): Mechanical properties of a single stress fiber from cultured smooth muscle cells. Abstract No. 1198, *Proc. 2004 Annual Fall Meeting*, BMES, Philadelphia, PA, Oct. 13-16, 2004, CD-ROM.

Sipkema, P.; van der Linden, P. J. W.; Westerhof, N.; Yin, F. C.-P. (2003): Effect of cyclic axial stretch of rat arteries on endothelial cytoskeletal morphology and vascular reactivity. *J Biomech*, vol. 36, pp. 653-659.

Stamenović, D. (2005): Effects of cytoskeletal prestress on cell rheological behavior. *Acta Biomaterialia*, vol. 1, pp. 255-262.

Takemasa, T.; Sugimoto, K.; Yamashita, K. (1997): Amplitude-dependent stress fiber reorientation in early response to cyclic strain. *Exp Cell Res*, vol. 230, pp. 407-410.

Wang H-B, Dembo M, Wang Y-L. (2000): Substrate flexibility regulates growth and apoptosis of normal but not transformed cells. *Am J Physiol Cell Physiol*, vol. 279, pp. C1345-C1350.

Wang, J. H.-C.; Goldschmidt-Clermont, P.; Yin, F. C.-P. (2001): Contractility affects stress fiber remodeling and reorientation of endothelial cells subjected to cyclic mechanical stretching. *Ann Biomed Eng*, vol. 28, pp. 1165-1171.

Wang, N.; Stamenović, D. (2000): Contribution of intermediate filaments to cell stiffness, stiffening and growth. *Am J Physiol Cell Physiol*, vol. 279, pp. C188-C194.

Wang, N.; Tolić-Nørrelykke, I. M.; Chen, J.; Mijailovich, S. M.; Butler, J. P.; Fredberg, J. J.; Stamenović, D. (2002): Cell prestress. I. Stiffness and prestress areclosely associated in adherent contractile cells. *Am J Physiol Cell Physiol*, vol. 282, pp. C606-C616.

Wille, J. J.; Ambrosi, C. A.; Yin, F. C.-P. (2004): Comparison of the effects of cyclic stretching and compression on endothelial cell morphological responses. *ASME J Biomech Eng*, vol. 126, pp. 545-551.