

Do Cells Sense Stress or Strain? Measurement of Cellular Orientation Can Provide a Clue

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ABSTRACT We predict theoretically the steady-state orientation of cells subject to dynamical stresses that vary more quickly than the cell relaxation time. We show that the orientation is a strong function of the Poisson's ratio, ν , of the matrix when cell activity is governed by the matrix strain; if cell activity is governed by the matrix stress, the orientation depends only weakly on ν . These results can be used to differentiate systems in which the strain or the stress determine the setpoint for the mechanosensitivity of cells.

Received for publication 19 November 2007 and in final form 28 December 2007.

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Biological cells exert forces and respond to the stresses or strains found in the environment (1) by remodeling the stress fibers and traction forces to maintain a tactile setpoint in the adjacent matrix (1–5). However, whether the cell mechanosensitivity (and hence its active response and setpoint) is controlled by the stress (force) in the extracellular matrix or by the strain (deformation), has not yet been resolved.

Measurements of the traction forces between cells and the substrate by Saez et al. (5) suggest that the cellular forces are governed by the deformation of the matrix and that the cell maintains an optimal strain in the matrix (6). However, Freyman et al. (4) have measured the macroscopic contraction of the substrate and conclude that the force is maintained at an optimal value.

In this letter, we predict cell orientation under cyclic stress as a function of the Poisson's ratio of the matrix (that can be varied experimentally (7,8)). We suggest that measurements of this effect can identify whether the controlling factor of cellular activity is strain or stress. In our theory, we extend our recent model (9) that combines both active and mechanical forces to explain the puzzling observation of different cellular orientation for static and dynamically applied stresses.

In our theory, each cell is modeled by an anisotropic force dipole tensor (10), which is the product of the oppositely directed forces exerted by each end of the stress fibers and their separation distance. For simplicity, we focus on cells that have bipolar morphologies, e.g., muscle cells and fibroblasts where the forces and the relative separations are both in the direction of the long axis of the cell, taken to be in the \hat{z} direction; the force dipole is thus written $P_{ij} = P\delta_{iz}\delta_{jz}$.

Motivated by experiments ((2–5), and S. Jungbauer and R. Kemkemer, unpublished), we assume that the cell tends to readjust its contractile activity by reorganizing the cytoskeleton to maintain a tactile setpoint, either an optimal strain, U^* , or an optimal stress, P^* , in the adjacent matrix. We assume that a cell whose axis is along z , regulates its contractile activity in response to the zz component of the local reaction strain or stress in the adjacent matrix. The relevant compo-

nents of the local reaction strain, U_{zz}^R , and reaction stress, σ_{zz}^R in the matrix at the long edge of the needlelike cell, due to the force dipole, are (see Supplementary Materials)

$$\begin{aligned} U_{zz}^R &= -P(1 + \nu)/(a^3 \pi E), \\ \sigma_{zz}^R &= -(2 - \nu)P/((1 - \nu)2\pi a^3), \end{aligned} \quad (1)$$

where a denotes the cell size, E the Young's modulus, and ν the Poisson's ratio of the matrix. The stress and strain represent a stretch in the adjacent matrix since the cellular contractile dipole, P , is negative.

The cell is subjected to forces that arise from the mechanical stresses of the matrix, as well as forces that are due to the activity of the cell that includes all the internal processes within the cell that regulate its contractile activity by reorganizing the focal adhesion and actin stress fibers. This gives rise to internal cellular forces that reestablish the optimal setpoint condition. Below, we treat separately the cases in which the cell activity is regulated by the stress or strain in the matrix. However, in both these cases, the matrix forces can be derived from the elastic deformation energy of the medium that includes the interactions of the dipoles with an external uniaxial stress, σ_a , applied at an angle θ relative to the cell axis. The total mechanical energy is written (see Supplementary Material for a review) as

$$F_m = P^2 \alpha(\nu)/(2\pi a^3 E) + P\sigma_a((1 + \nu)\cos^2 \theta - \nu)/E, \quad (2)$$

where $\alpha(\nu) = (1 + \nu)[15 + 2\nu(-13 + 8\nu)]/15(\nu - 1)^2$.

STRAIN AS THE SETPOINT

In this section, we assume that the forces due to cell activity act in a manner that the total local reaction strain is

Editor: Michael Edidin.

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doi: 10.1529/biophysj.107.126060

maintained at the optimal strain value, U^* . We begin by examining the case where the cellular forces dominate and then include the effects due to the matrix forces.

The component of the applied strain along the cell axis in the adjacent matrix, due to the external stress, is $U_{zz}^a = (1/E)(\sigma_a((1+\nu)\cos^2\theta - \nu))$. The optimal strain condition in the matrix is achieved when sum of the local reaction strain due to the contractile cell and the component of the external applied strain along the cell axis is equal to the optimal strain, i.e., $(U_{zz}^R + U_{zz}^a) = U^*$. Any deviation from this optimal strain condition gives rise to internal cellular forces that are derived from the variation of an effective free energy (11) written as

$$F_{c,n} = (\chi_s/2)(\sigma_a((1+\nu)\cos^2\theta - \nu)/E - P(1+\nu)/(a^3\pi E) - U^*)^2, \quad (3)$$

where χ_s is the measure of cell activity and has the dimension of energy. We rewrite the energies in dimensionless units: $f_{c,n} = F_{c,n}/(\chi_s U^{*2})$. The scaled local strain u_c due to the dipole P is defined as $u_c = P/(E'U^*)$, where $E' = E\pi a^3$ is the effective elastic energy. We also redefine the energy of the applied stress using $P_a = \sigma_a\pi a^3$; P_a is translated into a strain by defining $u_a = P_a/(E'U^*)$; u_a is the applied strain scaled by U^* . The dimensionless parameter, $c_s = E'/\chi_s$, is a measure of the competition between the forces due to cell activity and those due to the matrix elasticity.

We now predict the cellular orientation, θ , in the presence of dynamic stress, $\sigma = \sigma_a(1 - \cos\omega_a t)$. The dimensionless frequency of the applied stress is $\omega = \omega_a\tau_R$, where τ_R is the cellular relaxation time (9). We first consider the case where active cellular forces are much larger than the matrix forces and the cell energy controls the dynamics. If the time variation, $1/\omega_a$, of the external cyclic stress is much faster than the cell relaxation time, the cell cannot instantaneously follow the external stress and the long time solution is calculated by averaging the forces, or equivalently the free energy (9,11) over a cycle. The average of the cell energy $F_{c,n}$ over a cycle is written in dimensionless units,

$$\langle f_{c,n} \rangle = \frac{1}{2}[u_a((1+\nu)\cos^2\theta - \nu) - u_c(1+\nu) - 1]^2 + \frac{1}{4}[u_a((1+\nu)\cos^2\theta - \nu)]^2. \quad (4)$$

The second term in Eq. 4 arises from the averaging of the external field over the cycle and adds a positive contribution to the total free energy, compared to the static case. The steady-state solutions (11) predict: $\theta^s = 0, (\pi/2), \cos^{-1}(\nu/(1+\nu))^{1/2}$. When $\nu > 0$, the matrix is stretched in the direction of the uniaxial stress and is compressed in the perpendicular direction. In between these two directions, there is an angle at which the strain is zero; this is the orientation chosen by the cell (the cosine term above). In this direction, there are no time-varying strains and the cell can achieve the optimal strain, with no dynamical frustration (9). When $\nu < 0$, the matrix is expanded in all directions and there is no direction of zero strain. The cell does its best and chooses the direction of the minimal strain,

perpendicular to the applied stress. This is verified by a stability analysis of the solutions. We note that our prediction of the orientation, θ^s , as a function of ν turns out to be the same as suggested by Schwarz and Safran (10); however, our prediction is based on a dynamical model that takes into account both the cell and matrix forces (9). We now show how the matrix forces modify the dynamics and the steady-state response.

For high frequency, external dynamic stress, we now include the matrix forces and average the total effective free energy including both Eqs. 2 and 3, over a cycle. The cell energy, proportional to u_a^2 , competes with the matrix forces (that arise from the term in the total, scaled free energy that is proportional to $c_s u_a$) that drive the cell to parallel orientation. Thus, the ratio (c_s/u_a) determines the steady state values of the dipole magnitude and direction (11). We find, $\theta = 0, (\pi/2), \cos^{-1}[(\nu/(1+\nu))^{1/2} + (c_s/u_a)(1 + \alpha(\nu) + \nu)/((1+\nu)^2 \sqrt{\nu(1+\nu)})]$ in the limit of $(c_s/u_a) \ll 1$. A stability analysis shows that if the applied field is very small, then the driving force due to the cell activity is negligible and the predicted stable cellular orientation is $\theta^s = 0$, in the parallel direction. Indeed, there exists a threshold value of the applied field above which cells start orienting, consistent with the experimental observations. In addition, we have carried out a detailed numerical calculation (9) of the steady-state orientation for the general case where (c_s/u_a) is not necessarily small. Fig. 1 shows the steady-state cellular orientation, θ , as a function of the Poisson's ratio, ν . We show the solution for three different values of $c_s = 0.001, 0.01, \text{ and } 0.1$ for a scaled applied strain $u_a = 0.5$; increasing c_s represents an increase in the matrix forces. For values of c_s close to one or greater, for u_a fixed, the cell orientation is parallel to the applied stress. Comparing the steady-state orientation set by the cellular forces alone (*solid line* in Fig. 1) with the orientation due to the total of both the cellular and matrix forces (Fig. 1), we see that for $\nu > 0$, the matrix forces drive the steady-state orientation to smaller angles.

STRESS AS THE SETPOINT

We now predict the orientation for dynamically varying stress for the case in which the cell regulates its cytoskeleton in response to the total stress in the adjacent matrix; this is the case where the cell maintains an optimal stress, P^* , as a setpoint. The approach is similar to that in the previous section. The effective energy due to cell activity is written as

$$F_{c,s} = (\chi/2)(\sigma_a \cos^2\theta - \beta(\nu)P/(\pi a^3) - P^*)^2, \quad (5)$$

where $\beta(\nu) = (2 - \nu)/2(1 - \nu)$, and χ is a measure of cell activity and has the dimensions of an inverse energy.

We first consider the case where the dynamics is dominated by the internal, cellular activity. As above, we average the cellular free energy (Eq. 5) over a cycle. The local stress due to the dipole, P , and the applied stress $P_a (= \sigma_a\pi a^3)$ are scaled as $P = pP^*$ and $P_a = p_a P^*$, where p and p_a are dimensionless. We define the dimensionless parameter that measures the relative strength of the matrix and cellular forces: $c = 1/(\chi E')$.

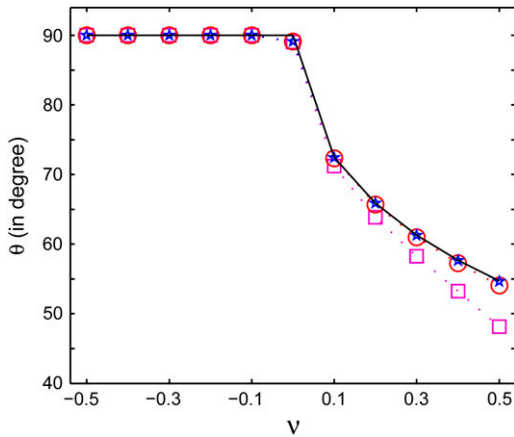


FIGURE 1 Numerical calculations of the cellular orientation, θ , as a function of the Poisson's ratio, ν , of the matrix for the case of strain as setpoint for three different values of $c_s = 0.001$ (\star), 0.01 (\circ), and 0.1 (*square*) with applied strain magnitude $u_a = 0.5$ and frequency $\omega = 10$ (scaled units). The solid line shows the analytical result discussed in the text.

For time-varying, external uniaxial stresses that vary more quickly than the cellular relaxation time, the cell cannot instantaneously follow the quickly varying, external stress (9). Because of this frustration, the cell orients in the perpendicular direction in which there is no time-varying stress and the cell can easily establish its dipole strength to match that of the setpoint. This is seen mathematically by solving for the steady-state solutions of Eq. 5, averaged over a cycle, as above. The resulting cellular orientation is independent of the Poisson's ratio of the matrix. This is in contrast to the case where the setpoint is determined by strain where the steady-state cellular orientation depends on ν , as given above. Thus, measurements of orientation angle as a function of Poisson's ratio of the matrix can differentiate the cases of optimal stress or an optimal strain as the regulator of mechanosensitive cellular activity. As above, we can include the effect of the matrix forces. The steady-state orientation, θ^s , is then given by $\theta^s = 0, \pi/2, \cos^{-1}[(1/\beta)\sqrt{2(c/p_a)} \sqrt{\alpha + \beta(1 + \nu(1 + p_a))}]$, for $c/p_a \ll 1$. The numerical results for the steady-state orientation are plotted in Fig. 2 for three different values of the parameter c that characterizes the strength of the matrix forces. As we found analytically for the case where the matrix forces are negligible, the angle has only a weak dependence on the Poisson's ratio compared with the case in which the strain is the setpoint. For large values of the matrix forces, the orientation tends to be parallel, as discussed above.

Our quantitative theoretical predictions of cell orientation under dynamically varying, external stress as a function of the Poisson's ratio of the matrix cannot yet be compared with the literature since such studies have not yet been carried out. However, our theory suggests a protocol that can be used in experiments by which cells that are regulated by matrix stress and cells that are regulated by matrix strain can be distinguished.

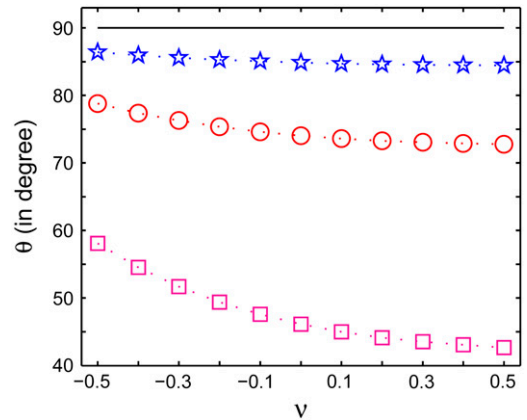


FIGURE 2 Numerical calculation of the steady-state cellular orientation, θ , as a function of the Poisson's ratio, ν , of the matrix for the case of stress as setpoint for three different values of $c = 0.001$ (\star), 0.01 (\circ), and 0.1 (*square*) with applied stress magnitude $p_a = 0.5$ and frequency $\omega = 10$ in scaled units. The solid line shows the orientation predicted analytically for negligible matrix forces. For $c \sim 1$ (keeping p_a fixed), $\theta \approx 0$.

SUPPLEMENTARY MATERIAL

To view all of the supplemental files associated with this letter, visit www.biophysj.org.

We thank A. Buxboim, L. J. Gibson, N. Gov, S. Jungbauer, R. Kemkemer, B. Ladoux, U. Schwarz, and P. Silberzan for useful comments.

We thank the Israel Science Foundation for support.

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- We note that cell is a highly nonequilibrium system and that the effective free energy used to derive the forces is not the thermodynamic free energy of the system.