

Dynamics of cell orientation

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Many physiological processes depend on the response of biological cells to mechanical forces generated by the contractile activity of the cell or by external stresses. Using a simple theoretical model that includes the forces due to both the mechanosensitivity of cells and the elasticity of the matrix, we predict the dynamics and orientation of cells in both the absence and presence of applied stresses. The model predicts many features observed in measurements of cellular forces and orientation including the increase with time of the cellular forces in the absence of applied stress and the consequent decrease of the force in the presence of quasi-static stresses. We also explain the puzzling observation of parallel alignment of cells for static and quasi-static stresses and of nearly perpendicular alignment for dynamically varying stresses. In addition, we predict the response of the cellular orientation to a sinusoidally varying applied stress as a function of frequency.

Understanding the active response of biological cells to mechanical forces is important for the rational design of artificial tissues, cellular self-assembly, muscle growth, wound healing and angiogenesis^{1–5}. Experiments on tissue cells, including fibroblasts, smooth-muscle cells, endothelial cells and stem cells have shown that these cells actively sense the mechanical properties of their environment and respond to the stress in the matrix by remodelling the actin cytoskeleton and other cellular processes. This feedback, in turn, induces changes in their contractile forces, migratory activity and orientations^{6–11}. A ubiquitous and puzzling experimental observation is that cells respond differently to static and dynamically varying strains. For static or quasi-static strain, cells align parallel to the direction of applied strain^{12,13}, whereas for cyclic strain, cells align away from the direction of the applied stretch; for high frequencies (~ 1 Hz), cells align nearly perpendicular to the strain direction^{10,11,14–20}. Here, we present a simple theoretical model that combines cell activity and the elastic forces exerted by the matrix on cells to explain several long-standing, universal and puzzling experimental results^{7,10–20}, including (1) the reduction in force exerted by cells in strained matrices (2), the parallel alignment of cells in response to static forces and (3) the nearly perpendicular alignment of cells in response to cyclically varying stresses. In addition, we predict how cell orientation varies as a function of the frequency of cyclically varying stresses and identify the various timescales that are involved.

Our model idealizes a stationary adhering cell, in a coarse-grained picture, as a pair of equal and oppositely directed contraction forces^{21,22}. This contractile force pattern is generated by active interaction of the myosin II molecular motors with the actin cytoskeleton resulting in stress fibres that connect focal adhesions on opposite sides of the cell^{21–25}. In general, each cell is modelled by an anisotropic force dipole tensor,

$$P_{ij} = l_i f_j = (l \mathbf{f}) n_i n_j = P n_i n_j;$$

with force components in all directions, where \mathbf{f} denotes the force exerted by the stress fibres and \mathbf{l} denotes the vector connecting

the focal adhesions. Here, the magnitude of the dipole strength for contraction dipoles $P < 0$ is typically $\simeq 10^{-11}$ J (ref. 21). In our model, for simplicity, we focus on needle-like cells (for example, muscle cells and often fibroblasts exhibit bipolar morphologies) for which the force, \mathbf{f} , and the vector connecting the focal adhesions, \mathbf{l} , are both in the direction of the cell axis. We shall take the instantaneous alignment of a needle-like cell to be in the z direction so that for these cells the force dipole has a single diagonal component: $P_{ij} = P \delta_{iz} \delta_{jz}$. Furthermore, we consider a single cell, appropriate to a dilute system; cell interaction can be taken into account via an ‘effective modulus’ of the system as shown in ref. 26.

THEORETICAL MODEL OF ACTIVE FORCE DIPOLES

In our theory, cells are modelled as elastic force dipoles that can change their contractile activity and orientation (that is, both the magnitude and direction of the dipole respectively) by reorganizing the focal adhesions and stress fibres in response to external forces. However, this reorganization can only occur if the temporal variation of the force is slower than the time required for the focal adhesions and stress fibres to reform. As shown below, this is the origin of the drastically different responses to static and dynamic external strains.

In experiments, it has been observed that cells exert forces in response to stresses in the matrix along the direction of the focal adhesions^{6,25}. Moreover, measurements of the forces exerted by cells suggest that the cell remodels its stress fibres and focal adhesions to maintain a constant local stress (a constant ‘endogenous’ matrix tension⁷; other experiments suggest an endogenous matrix strain that is sensed by cells^{1,27}). Cells taken from solution and placed in a gel establish a contractile force as time increases, reaching a steady-state value at long times and the further application of an external strain results in a decrease in the average contractile force exerted by the cells⁷. This suggests that cells regulate their contractile activity to maintain an optimal local force in the matrix in the presence of external stress. Motivated by these experiments, we assume that for needle-like cells oriented along the z direction this regulation is controlled by the z component of the local force

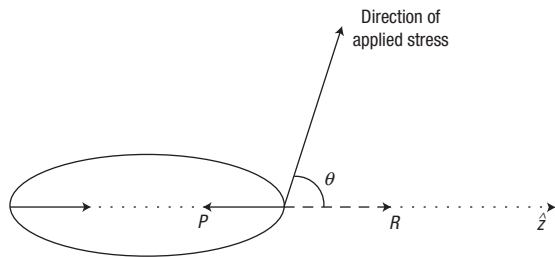


Figure 1 An illustration of the instantaneous position of a needle-like cell oriented along the z axis. The reaction stress, R , in the matrix arises from contractile activity of the cell, characterized by the force dipole, P .

in the matrix adjacent to the cell. This local reaction force to the contraction dipole of the cell, $P < 0$, is in the positive z direction and is proportional to $-P\hat{z}$.

In our reference frame shown in Fig. 1, θ is the orientation angle of the externally applied stress, σ_{ij} , relative to the instantaneous cell axis, \hat{z} . The force in the matrix in the direction along the cell axis is proportional to $\sigma \cos^2 \theta$, where σ is the magnitude of the applied stress²⁸. (The externally applied stress, σ_{ij} , has, in general, components in all directions. The relationship between the stress and the force results in one factor of $\cos \theta$ as the force is the dot product of the stress with the outward normal, $f_i = \sigma_{ij}n_j$. In addition, the evaluation of the component of the force in the direction of the cell axis (taken to be the z direction) involves a further dot product of the force with that direction, that is, $f_z = \mathbf{f} \cdot \mathbf{n}_z$ where $\mathbf{n}_z = n_z \hat{z} = \cos(\theta)\hat{z}$; \hat{z} is the unit vector along the z direction, that is, along the instantaneous cell axis. This brings in a further factor of $\cos \theta$. The overall dependence on $\cos^2 \theta$ is required by symmetry as the needle-like cell of our model cannot distinguish θ and $(\pi - \theta)$.) We define $P_a = \sigma\pi a^3$, to have dimensions of energy, where a is the cell size; this notation allows us to combine the external stress and dipole strength with the same units. The component of the total local stress in the matrix along the cell axis consists of the reaction stress, R , proportional to $-P$ and the applied external stresses if any. (We derive the local reaction stress in the matrix just outside the cell from the Green's function²⁹ as $R = -\beta(\nu)P/\pi a^3$, where $\beta(\nu) = (2 - \nu)/2(1 - \nu)$ and ν is Poisson's ratio. For simplicity of presentation the displayed formulae in the paper are for the case of $\nu = 0$.) In our model, cells readjust their contractile activity to maintain an optimal stress, σ^* , in the matrix whose force component in the \hat{z} direction is proportional to a quantity we define as $P^* = \sigma^*\pi a^3$, where again P^* has units of energy; this depends on the active-cell properties. The optimal total local stress in the matrix is achieved when $(-P + P_a \cos^2 \theta) = P^*$; that is, the sum of the reaction stress in the matrix to the contractile activity of the cell and the external stress is equal to the optimal stress, as in Fig. 1. Any change in this condition will result in the development of cellular forces that will tend to re-establish this condition. Even the establishment of a steady state is a dissipative process owing to the saturation of focal adhesions by actin–myosin contractility. The steady state is maintained only as long as there is sufficient ATP. For mathematical convenience, these forces can be derived from variations of an effective free energy, F_{cell} , that accounts for all of the local processes within the cell that establish a response to its local environment. Thus,

$$F_{\text{cell}} = \frac{1}{2} \xi (P_a \cos^2 \theta - P - P^*)^2;$$

where ξ is a measure of cell activity (with dimensions of an inverse energy) and is related to the tendency of the cell to reorganize

its focal adhesions and stress fibres. This occurs when the total local stress in the matrix deviates from the optimal stress P^* . The energy invested by a cell to re-establish the optimal stress P^* in the matrix is proportional to ξ . We note that this term alone does not uniquely determine the magnitude and the orientation of the force dipole in the steady state; it provides only a single constraint that involves both.

For mathematical simplicity and in accord with experiments on muscle cells and fibroblasts, we have assumed that the needle-like cells regulate their contractile activities with the stress fibres along the cell axis so that both \mathbf{f} and \mathbf{l} are in the z direction. However, for cells having more isotropic morphologies, the focal adhesions and stress fibres may orient in several directions. In these cases, our theory can be generalized by considering all of the dipolar components, and not only the 'zz' component. In most cases, the forces exerted by the stress fibres, \mathbf{f} , are parallel to the vector, \mathbf{l} , that connects focal adhesions, and the dipole tensor has only diagonal components. In these cases, the cell energy, F_{cell} , can be generalized as $F_{\text{cell}} \propto \sum_i (P_{ii}^a - P_{ii} - P^*)^2$; where P_{ii}^a denotes the component of the force due to the applied stress along the i th direction. Cells regulate their contractile activities to maintain an optimal stress, P^* , in each of the x , y and z directions. It may also happen that cells regulate different optimal stresses, P_i^* , in different directions.

However, the state of the cell (the magnitude of the force dipole as well as its direction) is determined not only by the internal dynamics of the cell but also by the mechanical forces exerted by the matrix; these arise from both long-range strains due to the cellular force dipoles and strains due to external forces.

Now, in the absence of any external force, the elastic deformation energy of an infinite medium due to a single force dipole (see equation (4) of ref. 22) can be written as $U_{\text{self}} = P^2/2E\pi a^3$, where E is Young's modulus of the matrix; this is also termed the self-energy of the dipole. This expression is for a dipole with a zz component only and with an appropriate choice of the small-distance cutoff in the integration over the medium²²; this cutoff is proportional to the cell size, a . For simplicity, Poisson's ratio of the matrix is taken to be zero. In general, $U_{\text{self}} = \alpha(\nu)P^2/2E\pi a^3$, where $\alpha(\nu) = (1 + \nu)[15 + 2\nu(-13 + 8\nu)]/15(\nu - 1)^2$. U_{self} represents a positive energy contribution that gives rise to a force that tends to diminish the magnitude of the force dipole, but is independent of its direction. In the presence of tensile strain, $u_{ij}^a > 0$, the interaction energy of a force dipole with an external strain field is proportional to the product of the force dipole and the external strain²². This adds a negative energy contribution (as $P < 0$ and $u_{ij}^a > 0$) that is minimal when the dipoles are aligned parallel to the strain direction. This can be understood from the fact that prestrain expands the matrix, whereas cells that are parallel to the strain contract the matrix, thus reducing the overall strain. We write the total mechanical energy for a cell in a matrix as:

$$F_{\text{matrix}} = U_{\text{self}} + P_{ij}u_{ij}^a.$$

The variation of this energy gives rise to forces that act on the cell in addition to those that arise from cell activity as discussed above. The total force that acts on the cell is derived from the variation of the total effective free energy that includes both the cell energy and the interaction energy of the matrix with the force dipole written as:

$$F_t = \frac{\xi}{2} (P_a \cos^2 \theta - P - P^*)^2 + \frac{1}{2} \frac{P^2}{E'} + \frac{P_a P \cos^2 \theta}{E'},$$

where $E' = E\pi a^3$ and θ is the orientation direction of the externally applied stress relative to the cell axis. For simplicity of presentation, we have written the equations for the case

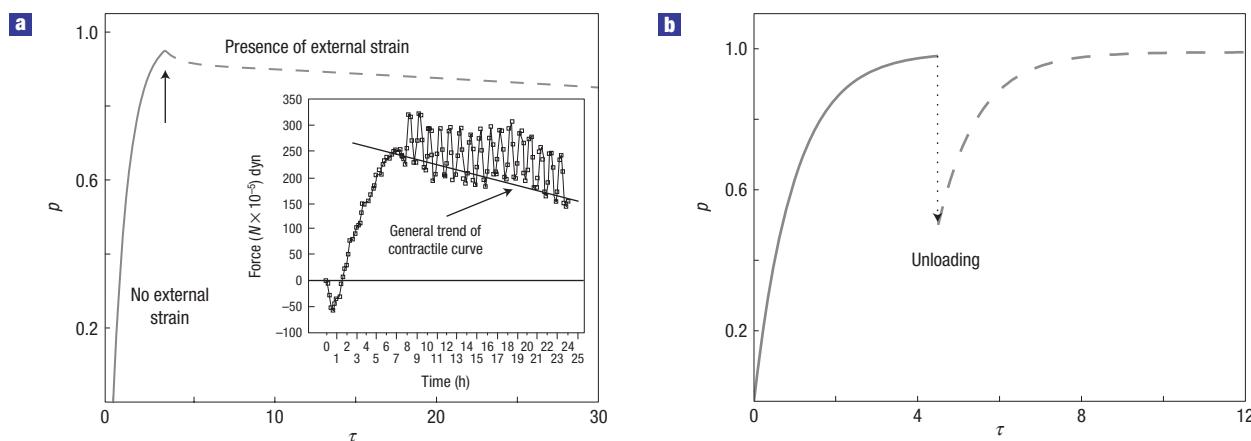


Figure 2 Time evolution of the strength of the force dipole in the presence of applied static stress. **a**, Absolute value of the dimensionless force dipole, p , as a function of scaled time, τ , in the absence of applied stress for times $\tau < 4$ (solid line). The parameter $c = 0.01$ and the initial value of p is taken to be zero corresponding to cells that initially exert no contractile forces. At $\tau = 4$, an external stress $p_a = 0.5$ is applied (shown by an arrow). The absolute value of the dipole decreases (dashed line) and saturates to a value of ~ 0.5 at long times. The inset shows experimental measurements: the medium is initially unstressed and at a time of 8 h an external nearly static stress is applied for 16 h (ref. 7). The (redrawn) inset is reprinted from ref. 7 with the permission of Wiley-Liss, a subsidiary of John Wiley and Sons Inc. **b**, A similar plot for unloading the matrix tension that occurs at $p_a = 0.5$, shows an increase of the absolute magnitude of the contractile force that restores the matrix tension to its earlier level.

where Poisson's ratio of the elastic matrix is taken to be zero. This is not an essential assumption. The general formula is $F_t = (\xi/2)(P_a \cos^2 \theta - P\beta(\nu) - P^*)^2 + (\alpha(\nu)/2)(P^2/E) + (1/E)P_a P[(1 + \nu) \cos^2 \theta - \nu]$, where ν is Poisson's ratio and $\beta(\nu)$, $\alpha(\nu)$ are mentioned above. The qualitative features are independent of ν as long as (ξE) is large.

We rewrite the free energy, F_t , in dimensionless units as $F = F_t/(\xi P^2)$. The local stress due to the dipole, P , and the applied external P_a are scaled as $P = pP^*$ and $P_a = p_a P^*$, where p and p_a are dimensionless. The constant 'endogenous' matrix stress, proportional to P^* , is thus scaled as unity. We define the dimensionless parameter $c = 1/(\xi E)$. This is a measure of the competition between the forces due to cell activity and those due to the matrix elasticity. Our theory can also be reformulated in terms of an 'optimal endogenous strain' as suggested in refs 1,27; the main qualitative features of different cellular orientations in the presence of static and dynamic stresses remain unchanged.

PREDICTIONS AND COMPARISON WITH EXPERIMENTS

The dynamical behaviour of the system relates the change in time of the dipole magnitude and orientation in a linear manner to the forces derived from the variation of the effective free energy discussed above³⁰:

$$\frac{dp}{dt} = -\frac{1}{\tau_p} \left(\frac{\partial F}{\partial p} \right); \quad \frac{d\theta}{dt} = -\frac{1}{\tau_\theta} \left(\frac{\partial F}{\partial \theta} \right). \quad (1)$$

Here, τ_p and τ_θ are the relaxation times for the readjustment of the magnitude and orientation, respectively, of the force dipoles and hence the reorganization times of the focal adhesions and stress fibres (order of tens of minutes⁹⁻¹¹). For simplicity in our numerical calculations below, we set $\tau_p = \tau_\theta = \tau_R$. However, the steady-state solutions we find do not depend on these timescales. For time-varying stresses, the results will be time dependent and we shall define the scaled time $\tau = t/\tau_R$ and frequency $\omega = \omega_0 \tau_R$, where ω_0 is the frequency of the applied stress.

Motivated by the experimental puzzles discussed above, we predict the response of cells for three experimentally relevant

scenarios: (1) in the absence of external stress, (2) in the presence of constant static stress and (3) in presence of time-dependent applied stress. Our model contains two dimensionless parameters, c (the inverse of the product of ξ and modulus E) and p_a (the dimensionless measure of the applied stress), that can be measured in experiments.

In the absence of applied stresses, the generalized force acting on the system is proportional to $\partial F/\partial p$, where $F = (1/2)(-p-1)^2 + (1/2)c p^2$. We find the time evolution of p (from, $dp/d\tau = -\partial F/\partial p$); $p(\tau) = (1/(1+c))[-1 + e^{-(1+c)\tau} (1 + p_0 + c p_0)]$, where p_0 represents the initial value of p . The steady-state value of $p = p_s$ is given from $dp_s/d\tau = 0$ and yields $p_s = -1/(1+c)$. This shows that the contractile activity of the cell, that is, the dimensionless force dipole, p , increases and eventually saturates to $1/(1+c)$, to maintain an optimal local force along the direction of the stress fibres. Note that the parameter c is inversely proportional to the elastic modulus, E , of the matrix, which implies that p increases with increasing rigidity of the matrix.

However, in the presence of static external strain, cells adjust their contractile activity to maintain the optimal local stress, σ^* , in the matrix. The experiments of ref. 7 show that once cells establish contractile forces in the matrix, a further application of applied strain results in a decrease of this force as shown in the inset of Fig. 2a. Our model accounts for this observation from the solution of equation (1). Figure 2a shows the predicted increase in the magnitude of the force dipole, p , in the absence of applied strain (shown by a solid line) followed by a decrease once the external strain field is turned on (shown by a dashed line) as observed in the experiment shown in the inset (see Fig. 4a of ref. 7). Moreover, once cells establish their optimal contractile forces in the matrix, a further release of the matrix tension by unloading results in an increase of the contractile force to restore the endogenous matrix tension to its earlier level. Thus, when the matrix tension is released by an applied unloading stress p_a (the force in the matrix in the direction along the cell axis is proportional to $(-|p_a| \cos^2 \theta)$ for unloading, whereas for loading it is $(|p_a| \cos^2 \theta)$), the cell contractile force, p ($p < 0$), increases such that $(-p + p_a \cos^2 \theta) = p^*$ is maintained (in dimensionless units $p^* = 1$). This restores the system to the optimal total local stress p^* ; this is also in agreement

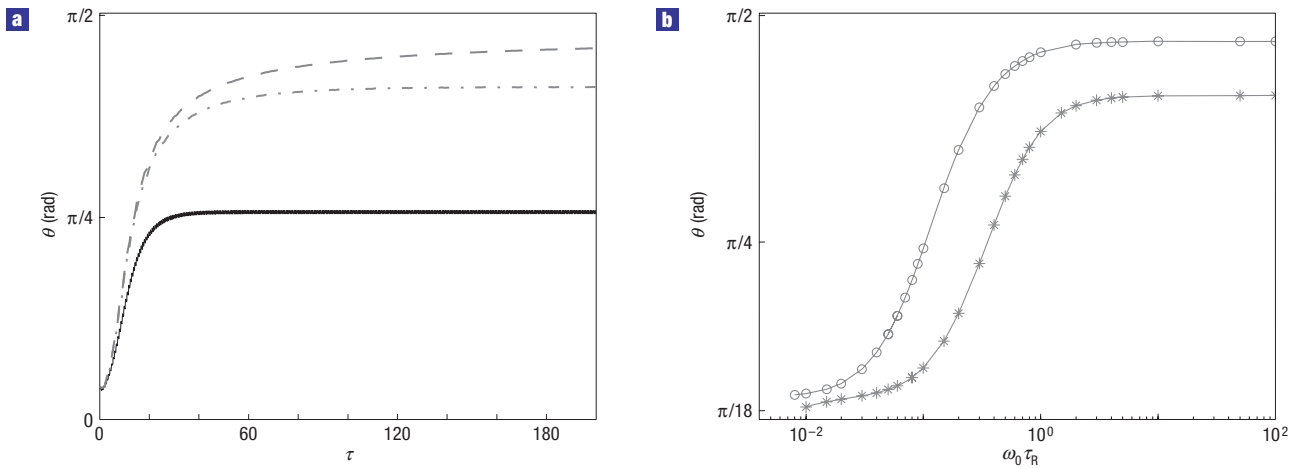


Figure 3 Time evolution of cellular dipole orientation in the presence of external cyclic stress. **a**, Time evolution of the cellular orientation, θ , as a function of the scaled time, τ , for three different values of $c = 0.001$ (dashed line), 0.01 (dashed-dotted line) and 0.1 (solid line) with applied stress magnitude $p_0 = 0.5$ and scaled frequency $\omega = 10$. Note that there are oscillations about the lines shown, but for large values of ω , the amplitudes of these oscillations are small and not visible on the scale shown. **b**, Cellular orientation, θ , as a function of the scaled frequency, ω ($= \omega_0 \tau_R$), of the applied stress with stress magnitude $p_0 = 0.5$, for $c = 0.01$ (asterisks) and $c = 0.001$ (circles). The parameter $c = 1/(\xi E')$, where ξ is a measure of the cell activity and E' is the elastic modulus of the matrix.

with what has been observed in the experiment (see Fig. 2 of ref. 7) shown in Fig. 2b. The theory predicts that, in general, the steady-state value of p is $p_s = (1/(1+c))(-1 + p_a \cos^2 \theta - c p_a \cos^2 \theta)$. This depends on the relative angle of the cell and the applied stress.

The steady-state value of the orientation can be derived from $\partial F/\partial \theta = 0$; this gives the force balance condition for static applied stress. A stability analysis shows that parallel cellular alignment is the stable orientation configuration as long as $p_a(3-c) < 2$. This implies, for instance, that for any value of c and $p_a \leq 1$ (that is, $\sigma \leq \sigma^*$, optimal endogenous matrix tension), the cells align parallel to the applied strain. A dynamical calculation of the cellular orientation, θ , from the coupled relaxation equation (1) in the presence of a static strain, shows that in steady state θ approaches ‘zero’, that is, the cell aligns along the direction of the constant applied strain as observed in experiment.

However, in the presence of dynamic cyclic external stress, $\sigma = \sigma_0(1 - \cos(\omega_0 t))$, the response of cellular alignment is quite different. If the time variation, $1/\omega_0$, of the applied stress is long compared with the relaxation time, τ_R , of the focal adhesions and cell rotation, the cells have sufficient time to readjust and reorganize. But if $\omega_0 \tau_R \gg 1$, the cells cannot follow the time-dependent strain. Instead, they can only respond to the time average of the sinusoidally varying stretch and then react accordingly. Therefore, to predict the long-time situation for cyclic strain for $\omega_0 \tau_R \gg 1$, we average $\sigma(\tau)$ in the effective free energy (or equivalently in the dynamical equations) over a cycle, the gradient of which derives the effective generalized force acting on the system. This average yields:

$$F = \frac{1}{2}(p_0 \cos^2 \theta - p - 1)^2 + \frac{c p^2}{2} + c p p_0 \cos^2 \theta + \frac{(p_0 \cos^2 \theta)^2}{4},$$

where p_0 is the amplitude of the cyclically varying stress in dimensionless units. The positive contribution of the further energy term, $(1/4)(p_0 \cos^2 \theta)^2$ (due to averaging the external field over the period), is maximal for parallel alignment ($\theta = 0$) but vanishes for perpendicular alignment; this is a simple way to see why the system is driven to nearly perpendicular orientation. Of course, the system is driven not

by the free energy, but by the forces. Thus, the steady-state solution, which can be derived by solving the coupled equations $dp/d\tau = 0$ and $d\theta/d\tau = 0$, predicts three possible orientations: $\theta_s \rightarrow 0, \pi/2, \cos^{-1} \sqrt{(4c/(1-c(-7+2c)))p_0}$. A stability analysis shows that the stable orientation is the near-perpendicular direction (the $\cos^{-1}(\dots)$ solution above) as long as c remains small, that is, when the elastic modulus of the matrix is large enough. Figure 3a shows the time dependence of the cellular orientation for cyclically varying stresses; the angle θ approaches a nearly perpendicular direction for small enough values of c . The numerical solution at long times as shown in Fig. 3a agrees with the analytical prediction for the cellular orientation as given above. Thus, our model predicts nearly perpendicular cellular alignment in response to a cyclically varying strain as observed in experiments^{10,11,14–20}, for cases in which the cell activity dominates the forces exerted by the matrix and where the product of the cyclic frequency (~ 1 Hz) and the cellular relaxation time (\sim minutes), $\omega_0 \tau_R$, is much greater than unity. If the matrix forces are dominant (either for small values of p_0 or values of $c \gg 1$), the cell may orient parallel to the applied force even in the dynamical case.

Our theory also allows us to predict the cellular orientation in the steady state as a function of the frequency of the applied sinusoidally varying strain as shown in Fig. 3b. We find an approximately two-state-like response of the cellular orientation. For very low frequencies (applicable to nearly static strains), cells have sufficient time to relax and therefore align nearly parallel to the external field. On the other hand, at high frequencies, cells opt for nearly perpendicular orientation to minimize the force acting on them due to the external strain. The physical origin of this effect is the frustration that exists in the high-frequency limit where the cell activity cannot instantaneously adjust to the magnitude of the applied force for cells in the parallel orientation. However, cells that are perpendicular to the applied force can organize their focal adhesions and stress fibres so that the local matrix stress reaches its optimal value, σ^* ; perpendicular cells do not ‘measure’ any local applied stress and are not frustrated. The balance of these frustration effects that arise from the cell activity and the matrix forces (that always drive the cell to the parallel orientation) result in a steady-state orientation that is nearly perpendicular for small

enough values of c . This frustration does not occur in the static case where the cell indeed can adjust its focal adhesions and the stress fibres so that the forces due to both the cell activity and the matrix elasticity balance.

Finally we note that, in general, we find that the cell forces increase with the matrix rigidity. Although this is in qualitative agreement with experiments^{2,7}, we note that the value of the local optimal stress, σ^* , may also be dependent on the matrix elasticity³¹. These effects are outside the theory presented above, whose focus is the prediction of cell orientation. It is worth mentioning that although our model of optimal stress/strain regulation is based on many experiments^{2,7}, it may not hold in all cases. Our theory is applicable to stationary mechanically active cells that established mature focal adhesions and are in mechanical equilibrium with the surrounding matrix. Indeed, for such systems, very recent experiments (S. Jungbauer, R. Kemkemer, private communication) show qualitative agreement with the ‘two-state’ behaviour predicted in Fig. 3b. However, experiments have yet to be carried out for different cell types and for different matrix stiffnesses. The functional form of the time and frequency dependence of such experiments can be directly compared with our predictions, whereas the numerical data can be used to extract values for the parameters σ^* and ξ of our theory. ξ can be obtained in dynamical stretching experiments from measurements of the cell orientation angle θ once the elastic modulus E' of the medium and the amplitude of the applied stress p_0 are known. Moreover, the cases of cells that are governed by optimal stress and cells that are controlled by optimal strain can also be distinguished by dynamical stretching measurements of the cellular orientation angle θ as a function of Poisson’s ratio, ν , in the limit of high frequencies of the applied stretch. Poisson’s ratio, ν , can be varied by changing the properties of the matrix.

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Competing financial interests

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