Dynamical theory of active cellular response to external stress

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We present a comprehensive, theoretical treatment of the orientational response to external stress of active, contractile cells embedded in a gel-like elastic medium. The theory includes both the forces that arise from the deformation of the matrix as well as forces due to the internal regulation of the stress fibers and focal adhesions of the cell. We calculate the time-dependent response of both the magnitude and the direction of the elastic dipole that characterizes the active forces exerted by the cell, for various situations. For static or quasistatic external stress, cells orient parallel to the stress while for high frequency dynamic external stress, cells orient nearly perpendicular. Both numerical and analytical calculations of these effects are presented. In addition we predict the relaxation time for the cellular response for both slowly and rapidly varying external stresses; several characteristic scaling regimes for the relaxation time as a function of applied frequency are predicted. We also treat the case of cells for which the regulation of the stress fibers and focal adhesions is controlled by strain (instead of stress) and show that the predicted dependence of the cellular orientation on the Poisson ratio of the matrix can differentiate strain vs stress regulation of cellular response.

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I. INTRODUCTION

As the basic unit of life, cells perform many specialized functions such as the encoding and decoding of genetic information, the synthesis and transport of molecules, and the maintenance of their own internal structure (homeostasis). Many of these biological processes involve cellular mechanical activity. The understanding of the mechanical activity of cells and its implications for intercellular as well as cell-extracellular matrix interactions is important for wound healing, muscle growth, tissue assembly, and development [1–8]. Numerous experiments indicate how cells sense their mechanical environment (e.g., its rigidity, and the presence of external strains). As living bodies, cells respond to these factors in an active manner (e.g., by actively adjusting their contractile activities) [9–12]. In addition it has been shown that cells remodel their cytoskeleton by reorganizing the stress fibers, adhesions, and traction forces to maintain a tactile set point in the adjacent matrix [1,13–16]. Many cell types show better developed stress fibers and focal adhesions (FA) when plated on rigid elastic substrates compared with floating (and elastically nonresistant) substrates [17]. It has also been observed that fibroblasts migrate from softer to stiffer substrates, orient themselves near material boundaries, and align in the direction of tensile strain [18]. In addition, there are experiments that show that cells respond differently to static or quasistatic strain (on the scale of many minutes) compared with rapidly varying cyclic strain (on the scale of 1 Hz). When the matrix in which the cells are embedded is subjected to a static or quasistatic strain, cells tend to orient along the direction of applied stress [19–23]. However, for rapidly varying strains, cells tend to orient away from the stress direction; for high frequency cyclic strain, cells align nearly perpendicular to the strain direction [24–33].

In this paper, we present a comprehensive theoretical study of the orientational response of cells in the presence of externally applied stresses as well as predictions for the influence of the surrounding elastic medium on the orientation dynamics. We address several long-standing experimental results including the parallel alignment of cells in response to static or quasistatic stresses and the nearly perpendicular alignment of cells in response to high frequency (~1 Hz) dynamic stresses. A brief report of some of the results was presented in [34] in which the dynamics was calculated numerically. Here, we present analytical results that predict the cell alignment dynamics; these results agree with the numerical calculations in the appropriate limits. In addition, we present calculations of the frequency-dependent relaxation time for the cells to reach their steady-state orientation. We find three distinct scaling regimes as a function of frequency of the cyclically varying applied stress. We also predict how the frequency-dependent cell orientation dynamics depends on the Poisson ratio of the matrix. Our theory suggests that the measurements of this effect can identify whether the cell mechanosensitivity is controlled by the stress (force) in the extracellular matrix or by the strain (deformation), a controversial issue—which is unresolved to date [15,16,35].

In Sec. II, we describe and explain our dynamical model in detail. Section III presents our predictions for cell orientation in the presence of both static and dynamic stresses, including the frequency dependence of the orientation. The second part of Sec. III focuses on the cellular relaxation time, i.e., the time required for the cell to reach its steady-state orientation, as a function of the frequency of the externally applied stretch. In Sec. IV, we show how the cellular response is affected by the variation of the Poisson ratio of the surrounding elastic medium. In the Appendixes we present further theoretical details including the elastic theory of dipoles, their self-energies (including a correction of results presented in [34,35]) and their interactions with external stresses, a derivation of the model free energy and dynamical equations that are based on symmetry arguments as well as an analytical solution for the frequency-dependent, cellular response to cyclically varying applied stresses.

II. THEORY: ACTIVE CELLS IN AN ELASTIC MATRIX

Recent experiments show that cells strongly respond to their mechanical environment. Anchorage-dependent cells establish numerous focal adhesions (FA) along their periphery and constantly probe the mechanical properties of their surroundings by assembling and disassembling focal adhesions. These FA are connected by actin stress fibers; the presence of myosin motors results in an internal tension of these stress fibers; the tension is transmitted to the extracellular matrix (ECM) via the FA. The FA act as mechanosensors and are an important component in the regulation of cell tension. In our model, we consider stationary, mechanically active cells that have already established mature focal adhesions and are in mechanical equilibrium with the surrounding matrix. The sum of the forces exerted by the focal adhesions, in a coarse-grained picture, are modeled as a pair of equal and oppositely directed contraction forces [36–38]. The local, focal adhesion structure is anisotropic since the forces that arise from the tension in the actin cytoskeleton tend to polarize the actin stress fibers and focal adhesions. Due to this anisotropic probing of the medium by the cells, the contraction dipoles can be described by an anisotropic force dipole tensor,

$$P_{ij} = l_i f_j = (lf) m_i n_j = P m_i n_j. \tag{1}$$

where $\vec{l}$ is a measure of the distance between the two equal but oppositely directed forces, $\vec{f}$, due to acto-myosin contractility. $\hat{n}$ and $\hat{m}$ are the unit vectors along the directions of $\vec{l}$ and the pinching force $\vec{f}$, respectively. Based on experimental measurements of forces exerted by cells, the typical magnitude of the dipole strength for contraction dipoles, $P<0$, is $\simeq 10^{-11}$ J [36]. In principle, the dipole tensor has force components in all directions. However, we shall specialize to the case where the force and the distance vectors are parallel so that $\hat{m}=\hat{n}$. Moreover, we focus on a single cell, appropriate to a dilute system. However, the results can be generalized to include interactions of many cells; cell-cell interactions result in "screening" of the external stress field that can be expressed by an effective "dielastic constant" as described in Ref. [39].

For simplicity, our model focuses on cells that show bipolar morphologies, e.g., muscle cells and fibroblasts. We assume the instantaneous alignment of such needlelike cells to be in the z direction; the force, $\vec{f}$, and the vector connecting the focal adhesions, $\vec{l}$, are then both along the cell axis (i.e., along the z direction) and the force dipole is

$$P_{ij} = P \delta_{iz} \delta_{jz}. \tag{2}$$

Appendix A reviews the elastic theory for a force dipole in an elastic medium and derives the stress, strain, self-energy, and interaction of a force dipole with an external stress or strain. These results are used in this section and in the remainder of this paper.

In our theory, we account for the fact that both the magnitude and the direction of the force dipole (that represents a contractile cell) are regulated by the cell which changes its contractile activity and orientation by reorganizing the focal adhesions and stress fibers in response to external forces. However, this reorganization can only occur if the temporal variation of the external force is slower than the characteristic time required for the focal adhesions and stress fibers to reform. As discussed below in detail, this is the origin of the very different responses of cells to static and quickly varying, time-dependent external stresses. Cells pull on their environment and via their mechanosensitivity, establish forces in response to stresses in the matrix along the direction of the focal adhesions [13,14]. Moreover, measurements of the forces exerted by cells in a strained matrix suggest that cells remodel their stress fibers and FA to maintain a constant total local stress (a constant, "endogenous" matrix tension as referred in Ref. [14]). Cells taken from solution and placed in a gel, establish a contractile force that eventually reaches a steady-state value at long times. Some experiments show that after this state is reached, the application of an external strain to the system, results in a decrease in the average contractile force exerted by the cells [14]. This suggests that cells readjust their contractile activity to maintain an optimal, local force in the matrix in the presence of external stress.

For simplicity, and to model needle-shaped cells, such as fibroblasts and muscle cells, we consider an elongated cell whose long axis is in the z direction. We assume that the focal adhesions and the stress fibers are also oriented in this direction and thus, the force dipole has only zz component. In addition, motivated by the experiments that show that cells regulate their contractile activity in response to the stress in the matrix, we assume that the cell maintains a set point that is determined by the response of the focal adhesions to the zz component of the stress in the matrix (both the force as well as surface normal are in the z direction). Since these adhesions are located at the tip of the cell we assume that their mechanosensitivity is regulated by the zz component of the stress right outside the "tip" of the cell. For a cell located at the origin, this "tip" is at $\vec{r}=(0,0,a)$ where a denotes the cell size.

Thus, for a force dipole, $P_{ij}=P\delta_{iz}\delta_{jz}$, located at the origin $[\vec{r}=(0,0,0)]$, the local reaction strain U^R_{zz} at $\vec{r}=(0,0,a)$ in the matrix can be derived from the Green's function using Eq. (A2) and Eq. (A7),

$$U^R_{zz} = -\frac{P(1+\nu)}{a^3 \pi E}, \tag{3}$$

where E is the Young's modulus and ν is the Poisson ratio of the matrix. Similarly, the other strain components are

$$U^R_{xx} = U^R_{yy} = \frac{P(1+\nu)}{4a^3 \pi E(1-\nu)}. \tag{4}$$

The corresponding local stress components are given (following Ref. [40]) as

$$\sigma_{ij} = \frac{E}{(1+\nu)}\left(u_{ij} + \frac{\nu}{1-2\nu} u_{ll}\delta_{ij}\right). \tag{5}$$

Because the focal adhesions tend to polarize and elongate along the force direction, we assume that the cell mechanosensors do not measure the angle-averaged stress but only the local component of the force along the cell major axis,

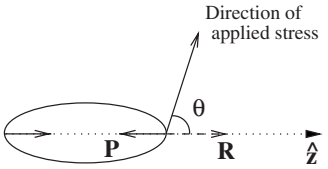

FIG. 1. An illustration of the instantaneous position of a needlelike cell oriented along the z axis. The reaction stress, R, in the matrix arises from contractile activity, $P<0$, of the cell.

just outside the cell. Thus, the zz component of the reaction stress, R, in the matrix just outside the cell and along the cell axis (in the z direction, which is the elongation direction of the mechanosensitive focal adhesions) is derived using Eq. (5),

$$R = \sigma_{zz}[\vec{r} = (0,0,a)] = -\frac{(2-\nu)P}{2(1-\nu)\pi a^3} = -\beta(\nu)\frac{P}{\pi a^3}, \quad (6)$$

where we define $\beta(\nu)=(2-\nu)/2(1-\nu)$. The total local stress in the matrix along the cell axis (positive z direction) is the sum of the reaction stress, proportional to $-P$, due to cell's own contractile activity and any applied external stresses. The reaction stress, R, represents a stretch in the adjacent matrix since the cell dipole $P<0$ is contractile.

As we have discussed before, we choose a coordinate system in which the instantaneous position of a needlelike cell is along the z axis and the angle of the externally applied stress, σ^a_{ij}, relative to z is θ, as illustrated in Fig. 1. The applied stress has, in general, components in all directions. The force in the matrix along the direction of the cell axis is $f_i=\sigma^a_{ij}n_j$ where n_j is the outward normal [40]. The component of the force along the z direction is given by $f_z=\vec{f}\cdot\hat{n}_z$. Thus, the component of the applied force along the cell axis is proportional to $\sigma^a\cos^2\theta$, where σ^a is the magnitude of the applied stress. One can also see this from symmetry considerations: The $\cos^2\theta$ dependence is due to the fact that the force dipole cannot distinguish the θ and $(\pi-\theta)$ directions. We define $P_a=\sigma^a\pi a^3$, which has dimensions of energy; this notation allows us to refer to the external stress and dipole strength using the same units.

In our model, the local activity of the cell adjusts the cell contractility by reorganizing the FA and stress fibers to maintain an optimal stress magnitude, $\sigma^\star$, in the adjacent matrix along the z direction. To convert this optimal stress to an energy, we define $P^\star=\sigma^\star\pi a^3>0$. The optimal total local stress in the matrix is achieved when the sum of the reaction stress [Eq. (6)] in the matrix, R, due to cell contractile activity and the external stress is equal to the optimal stress, P^*, i.e., $(R+P_a\cos^2\theta)=P^\star$. In the absence of any external stress, the optimum condition implies that $R=P^\star$. Any change in this condition will result in the development of internal forces within the cell that will tend to reestablish the optimal force condition. For mathematical convenience, these forces can be derived from variations of an effective free energy, $F_{c,s}$ that accounts for all of the local processes within the cell (e.g., reassembly of the FA and stress fibers as well as the effects of the myosin molecular motors) that establish the cellular response to its local environment. Thus,

$$F_{c,s} = \frac{1}{2}\chi\left(P_a\cos^2\theta - \frac{(2-\nu)}{(2(1-\nu))}P - P^\star\right)^2, \quad (7)$$

where χ is a measure of cell activity, (i.e., the tendency of the cell to reorganize its focal adhesions and stress fibers once the stress in the matrix is not at its optimal value) and has the dimensions of an inverse energy. However, the internal cell dynamics 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 the magnitude of P and its direction. A unique determination of both these factors can be derived if one includes the mechanical forces exerted by the matrix on the cell. In general, one could also consider stochastic forces that act on the cell, but these are outside the scope of the present work; some effects due to random forces are discussed in [39]. The mechanical forces are derived from the elastic deformation energy of the matrix. We thus include both the deformation energy of the elastic matrix arising from the long-range strains due to the cellular force dipoles as well as strains due to external forces.

For an applied stress, $\sigma^a_{ij}=\sigma^a n_i n_j$, whose corresponding strain in the matrix is u^a_{ij}, we calculate the strain field [40] along the cell axis in the z direction: $u^a_{zz}=\sigma^a[(1+\nu)\cos^2\theta-\nu]/E$. This is true in the dilute limit where the cell density is low enough that depolarization effects due to the reorientation of the surrounding cells can be neglected. We thus, write the mechanical energy of a cell in the presence of an external stress as (for detailed derivation see Appendix A)

$$F_m = \frac{1}{2}\frac{P^2}{E'}\alpha(\nu) + \frac{1}{E'}P_a P[(1+\nu)\cos^2\theta - \nu], \quad (8)$$

where $E'=E\pi a^3$, θ is the direction of the externally applied stress relative to the cell axis, and the constant $\alpha(\nu)$ depends on the regularization procedure for calculating the self-energy (see Appendix A). We note that the external stress can, in general, be frequency dependent and motivated by the experiments we consider cyclic stresses of frequency ω_a, $\sigma=\sigma^a(1-\cos\omega_a t)$.

The total generalized force that acts on the cell is derived from the variation of the total effective free energy, $F=F_{c,s}+F_m$, that includes both the cell energy and the matrix energy. We rewrite the free energy F in dimensionless units as $f=F/(\chi P^{*2})$. The local stress due to the dipole, P, and the applied external stress, P_a, are scaled by the optimal stress, $P^\star$, and we define $P=pP^\star$ and $P_a=p_aP^\star$ where p and p_a are dimensionless. We define the dimensionless parameter $c=1/(\chi E')$ which is a measure of the competition between forces due to cell activity and those due to the matrix elasticity. Thus, the total effective energy in dimensionless units is

$$f = \tfrac{1}{2}[p_a\cos^2\theta - p\beta(\nu) - 1]^2 + \tfrac{1}{2}cp^2\alpha(\nu) + cpp_a[(1+\nu)\cos^2\theta - \nu]. \quad (9)$$

In Appendix B, we show how the total effective free energy can be derived from symmetry arguments in which we require a free energy that is a scalar function of products of the stress tensor (for uniaxial stresses applied normal to the

cell boundary) and the two vectors that represent the cellular force and the long axis of the cell. We obtain terms similar to those written above, but without, of course, the physical distinction of mechanical forces, cell forces, stress set-point, etc.

III. THEORETICAL PREDICTIONS: DYNAMICS OF CELL ORIENTATION

A. Steady-state cellular response in the presence of static stress

As we have discussed in the preceding section, in the presence of an external applied stress, cells readjust their contractile activity to reestablish the optimal stress in the matrix. The net, generalized force acting on the system can be derived from the gradient of the effective free energy, f, from Eq. (9). For simplicity of presentation, we first study the dynamics for the case where the Poisson ratio of the elastic matrix is negligible (i.e., $\nu=0$).

We first minimize the free energy with respect to the magnitude of the dipole, p, to obtain a free energy that depends only on the cellular orientation angle θ,

$$f_\theta = \frac{c}{2(1+c\alpha)}[\alpha - 2(1+\alpha)p_a \cos^2\theta + (2+\alpha-c)p_a^2 \cos^4\theta], \tag{10}$$

where the constant $\alpha=\alpha(0)$ depends on the regularization procedure of the self-energy. To obtain the possible steady-state orientation angle, θ_s, we solve $\partial f_\theta/\partial\theta=0$ from Eq. (10), and find

$$\theta_s = 0, \quad \frac{\pi}{2}, \quad \cos^{-1}\sqrt{\frac{(1+\alpha)}{(2-c+\alpha)p_a}}. \tag{11}$$

A rigorous stability analysis shows that the parallel orientation will be stable as long as $p_a(2-c+\alpha)<(1+\alpha)$ in the presence of static applied stresses. For $\alpha=0$, the stability condition yields $p_a(2-c)<1$, while for $\alpha=1$, the stability condition turns out to be $p_a(3-c)<2$ (as discussed in Ref. [34]).

B. Cellular response to dynamic stress

In contrast to cell alignment in parallel with the direction of static stress, the orientational response in the case of dynamically varying external stress, $\sigma=\sigma^a(1-\cos\omega_a t)$, is quite different. If the time variation of the stress (proportional to the inverse of the external stress frequency ω_a) is long relative to the intrinsic relaxation time, τ_R, of the stress fibers and FA of the cell, cells have sufficient time to readjust and reorganize their FA and the stress fibers in order to maintain the optimal force condition in the adjacent matrix. In this case, cells can orient parallel to the applied stress.

However, if the frequency is high enough so that $\omega_a\tau_R \gg 1$, cells do not have time to instantaneously follow the rapidly varying, cyclic stress; they can only respond to the average value of the sinusoidally varying stretch and then react accordingly. Therefore, an analytical estimate of the long-time behavior, is given by calculating the average value of the free energy over a cycle, the gradient of which gives the effective generalized force acting on the system. (Averaging the free energy over a cycle is equivalent to averaging the effective force as shown in Appendix D.) This estimate agrees with our numerical calculations in the high frequency limit. The dynamic average of the free energy [derived from Eq. (9) for Poisson's ratio, $\nu=0$] over a period is

$$f^d = \tfrac{1}{2}(p_a\cos^2\theta - p - 1)^2 + \tfrac{1}{2}c\alpha p^2 + cpp_a\cos^2\theta + \tfrac{1}{4}(p_a\cos^2\theta)^2. \tag{12}$$

This gives rise to an additional energy term, $\frac{1}{4}(p_a\cos^2\theta)^2$, due to the averaging of the external field over a period. This contribution is maximal for parallel alignment but vanishes for perpendicular alignment. Thus, for high frequency applied stresses, our analytical estimate shows that the "dynamical frustration" of the cell drives the system to near perpendicular alignment with the applied cyclic stress.

As above, we first minimize the time-averaged free energy with respect to the dipole magnitude, p, to obtain the time-averaged free energy as a function of only the cellular orientation angle θ,

$$f^d_\theta = \frac{c}{2(1+c\alpha)}[\alpha - 2(1+\alpha)p_a\cos^2\theta + (2+\alpha-c)p_a^2\cos^4\theta] + \frac{1}{4}p_a^2\cos^4\theta. \tag{13}$$

The steady-state solution for the time-averaged dynamical case, is derived by solving the coupled equations $df^d/dp=0$ and $df^d/d\theta=0$. This predicts three possible orientations,

$$\theta_s \to 0, \quad \pi/2, \quad \cos^{-1}\sqrt{\frac{2c(1+\alpha)}{p_a\{1+c[(4+3\alpha)-2c]\}}}. \tag{14}$$

To specify the stable, steady-state orientation, we have performed a stability analysis that predicts that the stable orientation is the near perpendicular direction [the $\cos^{-1}(\cdots)$ solution above] as long as c remains small (i.e., the elastic modulus of the matrix is large enough).

The cell aligns nearly perpendicular to the applied stress for small enough values of c. For small values of c, where the cell activity dominates over the matrix forces, the contribution arising from the self-energy only changes the numerical values; the scaling behavior of the angle with c and the external stress magnitude p_a is still given by $\cos\theta \sim \sqrt{2c/p_a}$ for small values of c. However, for large values of c, the stable state is $\theta=0$; that is, when the mechanical forces dominate, the cell aligns parallel to the applied stress. As noted before, there can also be stochastic forces that induce a random alignment of the cells; our analysis can be generalized to include these forces, but the detailed treatment is outside the scope of this paper that focuses on the competition of the mechanical and cell-activity forces. We note that for finite nonzero values of c (corresponding to finite matrix rigidity) the cellular alignment is perpendicular only if the applied stress, p_a, is large enough [in terms of physical variables, $P_a \gg P^\star/(\chi E')$].

Our results show that the self-energy term does not change the qualitative behavior or the scaling with c (for small values of c, i.e., where the cell activity dominates the matrix forces), but only changes the numerical values of the plots. In the remainder of the paper we, therefore, neglect the self-energy term and set $\alpha=0$ for simplicity.

C. Relaxation dynamics

We predict the dynamical behavior of the system by calculating the forces that act to change both the magnitude and direction of the cellular force dipole. The dynamical equations specify a linear relationship between the temporal change of the dipole magnitude, p, and orientation, θ, and the generalized forces, derived from the variation of the effective free energy [41] using Eq. (9) for $\alpha=0$. In the simple relaxational model, the dynamical equations for the variables p and θ are given by (for a detailed derivation see Appendix C)

$$\frac{dp}{dt} = -\frac{1}{\tau_R}\frac{\partial f}{\partial p},$$

$$\frac{d\theta}{dt} = -\frac{1}{\tau_R}\frac{1}{p^2}\frac{\partial f}{\partial \theta}. \tag{15}$$

Here, τ_R is the relaxation time for the readjustment of the magnitude of the force dipoles. That is, in the absence of external stress, a cell placed from solution into an elastic matrix in which it develops stress fibers and FA, builds up its force from an initial value to a value determined by the optimal stress, in a time related to τ_R. For time varying applied stresses, the cell dynamics can have a complex time dependence and we shall define the scaled time $\tau=t/\tau_R$ and the scaled frequency $\omega=\omega_a\tau_R$, where ω_a is the frequency of the applied stress. In Appendix C, we compare the dynamical behavior obtained from Eq. (15) with a simplified treatment presented in [34,35]; the two compare very well, except at very large values of the applied stress.

Our theory involves only three model parameters two of which are measurable in experiments at zero stress: The cell activity χ, the optimal set point stress $P^\star$, and the internal cellular relaxation time τ_R. $P^\star$ and τ_R can be obtained by measuring the saturation cell force and the time required to reach saturation for cells taken from solution and placed in elastic medium [14,19]. The experimentally controllable variables are the magnitude P_a and the frequency ω_a of the external stress and the elastic modulus of the matrix E'. Thus, the cell activity, χ, remains as the only unknown parameter in the theory and it can be obtained in several different ways to check the consistency of the theoretical description by measuring the steady-state cell orientation angle, θ, in dynamical stretching experiments [34] and by measuring the characteristic relaxation time (as in Ref. [42]); we discuss this in detail in Sec. IV.

D. Analytical asymptotic solution and comparison with numerics

We now present an analytical calculation of the steady-state solution and, for simplicity, begin by rewriting the total free energy, f, of Eq. (9) for the case where the Poisson ratio of the elastic matrix is taken to be zero,

$$f_\phi = \tfrac{1}{2}(p_a\phi - p - 1)^2 + cp_a p\phi + \tfrac{1}{2}c\alpha p^2. \tag{16}$$

Here, we define $\phi=\cos^2\theta$ and the last term corresponds to the self-energy of the force dipole where the constant α depends on the regularization procedure for calculating the self-energy (see Appendix A). The dynamical behavior of the system is determined by the forces that act to change both the magnitude and direction of the cellular force dipole as discussed in the preceding section. Thus, the dynamical relaxational equations for the variables p and ϕ are derived following Eq. (15):

$$\frac{dp}{d\tau} = -\left(\frac{\partial f_\phi}{\partial p}\right), \quad \frac{d\phi}{d\tau} = -\frac{1}{p^2}4\phi(1-\phi)\left(\frac{\partial f_\phi}{\partial \phi}\right). \tag{17}$$

Since we focus on the case in which the temporal variation of the applied cyclic stress is periodic, the general solution of Eq. (17) can be represented by a suitable combination of sine and cosine functions. The general solution must be written as a sum over all modes,

$$p(\tau) = \mathrm{Re}\sum_{n=0}^{\infty} a_n e^{in\omega\tau},$$

$$\phi(\tau) = \mathrm{Re}\sum_{n=0}^{\infty} b_n e^{in\omega\tau}, \tag{18}$$

where $\omega=\omega_a\tau_R$ and $\tau=t/\tau_R$ is the dimensionless time variable. A treatment of the general solution loses the analytical simplicity. We, therefore, make the simplification (which is justified by comparison with the numerical results) that for high frequency cyclic stress, the solution is given by the sum of an in-phase and out-of-phase oscillation of the dipole at the fundamental frequency ω (as well as a constant term). In this approximation, we write

$$p(\tau) = a_0 + a_1 \sin\omega\tau + a_2\cos\omega\tau,$$

$$\phi(\tau) = b_0 + b_1 \sin\omega\tau + b_2\cos\omega\tau. \tag{19}$$

The Fourier coefficients a_0, a_1, a_2 and b_0, b_1, b_2 can be found by solving six algebraic equations obtained by substituting the solutions $p(\tau)$ and $\phi(\tau)$ in Eq. (17), multiplying by either unity, $\sin\omega\tau$, or $\cos\omega\tau$ appropriately and then integrating the equations over one period (0 to $2\pi/\omega$). We have ignored the higher harmonics in obtaining the solutions; this is a reasonable assumption for high frequency applied cyclic stresses. The equations that determine a_0 and b_0 are nonlinear. To obtain the other coefficients, we have expanded the Fourier coefficients in a series in $(1/\omega)$. This allows us to obtain the asymptotic analytical solution in the high frequency limit, $\omega\to\infty$, by solving the linearized equations (for a detailed derivation see Appendix E). In the high frequency regime, the steady-state solutions of the force dipole, p, and the orientation, θ, can be approximated by the constant Fourier coefficients a_0 and b_0, respectively.

We compare the asymptotic, analytical solutions with the numerical solutions of Eq. (17) for the high frequency re-

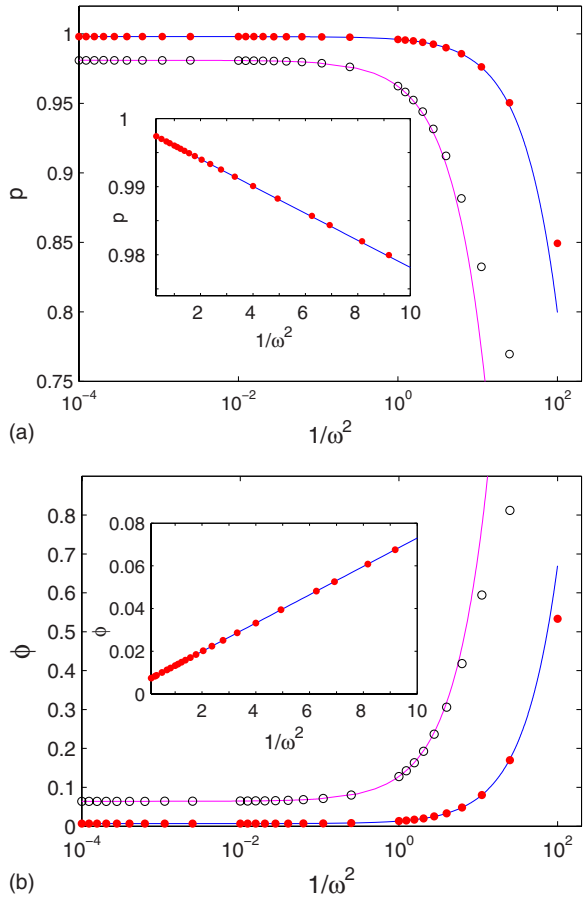

FIG. 2. (Color online) Comparison of the analytic and numerical predictions for the magnitude of the force dipole, p, and the cellular orientation, $\phi=\cos^2\theta$, as a function of $1/\omega^2$ where ω is the frequency of the external, cyclic stress. Numerical plots are shown by the points for two different values of $c=0.001$(•) and $c=0.01$(◦) for applied stress magnitude $p_a=0.3$ and the corresponding analytical solutions are shown by the solid continuous curves. The inset shows that the asymptotic steady-state solutions vary linearly with $1/\omega^2$ for high frequencies.

gime of cyclic stress. Figure 2 shows the comparison of the analytical and numerical solutions for the force dipole, p, and its orientation, $\phi=\cos^2\theta$, as a function of $1/\omega^2$ for two different values of $c=0.001$ and $c=0.01$ keeping the applied stress magnitude fixed at, $p_a=0.3$ and $\alpha=0$. The analytical approximation matches the numerics quite well for high frequencies, i.e., when the product of the applied frequency and the cellular relaxation time is greater than unity ($\omega_a\tau_R>1$). For high frequency cyclic stretch, cells do not have sufficient time to relax and are mainly driven by the time scale of the external periodic force. The applied stretching frequency thus determines the cell response and this is why the first Fourier mode is a good approximation for the dynamics. Moreover, since the orientation does not depend on the self-energy and also because we focus on systems in which the cell activity forces dominate the elastic forces, we have shown the plots for $\alpha=0$ (as discussed before). However, the analytical solutions are given for any value of α in the limit of small values of c as

$$a_0 = -1 + \left(c(2+3\alpha) + \frac{1}{\omega^2} 2c(1+\alpha) \right),$$

$$b_0 = \frac{2c}{p_a}(1+\alpha)\left(1+\frac{1}{\omega^2}\right). \tag{20}$$

As seen from Fig. 2, in the low frequency regime, $\omega_a\tau_R<1$, the numerical solution begins to deviate from the analytical one; that is expected since the analytical prediction is only valid in the limit of high frequency. In the low frequency limit, cells have sufficient time to relax and therefore respond by reorganizing themselves in specific ways; in this case, the internal cellular relaxation time plays a crucial role in the dynamical response. Therefore, the general solution can no longer be approximated by a single harmonic [Eq. (19)] at the fundamental frequency of the applied stress. In the low frequency limit where the cell relaxation time dominates the time scale of the applied force, we must consider the higher harmonics to capture the more complex cellular motion given by the more general solution of Eq. (18). In the following section, we discuss this frequency dependence of the internal cellular relaxation in more detail.

E. Characteristic relaxation time of dynamic reorientation

In the presence of an external stretch, cells readjust their contractile activity and reorganize their cytoskeleton; the competition of the internal cellular relaxation with the dynamic driving force determines the resulting cell response. It is important to understand the interplay of these time scales to obtain insight into the molecular mechanisms that govern the cell-matrix interactions and hence the cellular processes. However, this detailed quantitative understanding has not yet been achieved. In this section, we predict how the characteristic relaxation time that characterizes the overall orientational response and its approach to steady state [43], varies with the frequency of the time-varying external stress.

We have carried out detailed, dynamical calculations by solving the coupled relaxation equations using [Eq. (15)]

$$\frac{dp}{d\tau} = -\frac{\partial f}{\partial p}, \quad \frac{d\theta}{d\tau} = -\frac{1}{p^2}\frac{\partial f}{\partial \theta} \tag{21}$$

to predict the characteristic relaxation time as a function of applied frequency. Here, the dimensionless time ($\tau=t/\tau_R$) is the ratio of the actual time and the internal relaxation time, τ_R, of the cell. The cyclic stress, which is the driving force for cell orientation creates an oscillatory response in the cell; these oscillations gradually decrease in amplitude and the angle reaches its steady-state value. We have fit the mean value of the orientation angle, $\theta(\tau)$, with the functional form $\theta_{fit}(\tau)=a-be^{(-\tau/\tau_c)}$ to extract the characteristic relaxation time, τ_c, for the cell orientation dynamics. This time is the time required for the angle to reach its steady-state value starting from some initial random configuration in the presence of a time-varying external stress, and a and b are constants that are fitting parameters.

We have carried out this analysis for a wide range of frequencies of the applied cyclic stress. Figure 3 shows how the characteristic relaxation time, τ_c, of the cell orientation

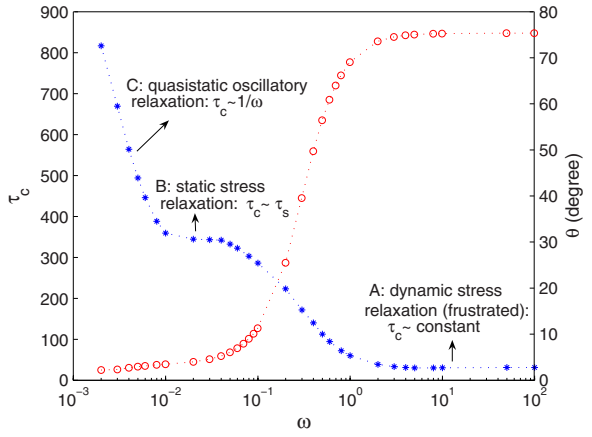

FIG. 3. (Color online) The characteristic relaxation time τ_c as a function of the dimensionless frequency $\omega(=\omega_a\tau_R)$ of the external cyclic stress for $c=0.01$ and applied stress magnitude $p_a=0.3$ (shown by ⋆, blue). The corresponding steady-state value of the cell orientation angle θ vs frequency ω is shown by the symbol ○ (red). (The small dots are just a guide to the eye.)

varies with the applied frequency, ω. Our theory predicts three distinct frequency regimes as shown in Fig. 3: (i) High frequency regime—this correspond to a regime where the characteristic relaxation time is asymptotically constant ($\tau_c \sim$ constant), i.e., independent of the frequency of the applied field; (ii) low frequency regime or quasistatic oscillatory relaxation where the relaxation time varies linearly with the time variation of the applied field ($\tau_c \sim 1/\omega$); (iii) intermediate frequency regime—in between regimes (i) and (ii), the characteristic relaxation time approaches the relaxation time that is characteristic of cell response to an external static stress. Below, we discuss the physics of these three regimes and their scaling relations in detail.

(i) High frequency regime: For high frequency external cyclic stresses, cells cannot instantaneously follow the rapidly varying stress and are therefore, unable to establish the optimal stress condition in the adjacent matrix. Therefore, these "frustrated" cells orient along the perpendicular direction which is the zero stress direction for the uniaxial applied stress, where they are unaffected by the applied cyclic stress and can attain homeostasis (their optimal stress).

For high frequencies ($\omega_a\tau_R \gg 1$), cells do not have sufficient time to relax; they can only respond to the time average of the cyclic stretch. The effective cellular forces that are governed by the time average of the applied stress can be derived from the gradient of the dynamically averaged (over one cycle) free energy of Eq. (13). Therefore, the effective cellular force no longer depends on the time variation of the cyclic stress and the characteristic time is approximately constant and independent of frequency as seen from Fig. 3 (regime A). This is also consistent with recent experimental observation [42]. An estimate of the characteristic time, τ_c, can be calculated analytically by deriving the time-averaged force from Eq. (13), $\partial f_\theta^d/\partial(\cos^2\theta) \approx \frac{1}{2}p_a^2(1+4c)\cos^2\theta$, approximated for small c and $\alpha=0$. If the cell energy (proportional to p_a^2) dominates over the matrix forces (proportional to cp_a), the value of τ_c scales as $1/p_a^2$. Thus, for example, the order of magnitude of τ_c can be estimated for the values of $c=0.01$ and $p_a=0.3$ as $\tau_c \sim 1/p_a^2 \sim 10$ whereas from the exact numerical calculations (shown in Fig. 3) τ_c turns out to also be of the same order ($\sim$30). This scaling relation for the order of magnitude of τ_c is also obeyed for the other values of the parameter p_a.

A more complete description of the scaling relations in terms of the physical variables (of which χ is not measurable from experiments at zero stress) predicts $\tau_c \sim \tau_R/\chi P_a^2/P^\star$ where χ has the dimension of an inverse energy; $P^\star$ and P_a have the dimensions of energy; τ_c and τ_R have dimensions of time (using the definitions discussed in Sec. II and where we assume that the magnitude of $P \sim P^\star$ is a steady state). Thus, knowing the cell relaxation time, τ_R, and the set point stress, $P^\star$, from experiments in the absence of stress and measurements of the characteristic time, τ_c, enable one to determine the cell activity, χ, as mentioned in Sec. III C. This can be checked for consistency by comparing the value of χ to that obtained from the cell orientation equation (14), $\cos\theta \approx \sqrt{[P^\star/(\chi P_a E')]}$.

(ii) Intermediate frequency regime: As the frequency of the applied cyclic stress decreases, the competition of the internal cellular relaxation time with the driving time scale of the applied stress plays a crucial role in governing the dynamics of cell response. At low frequencies, ($\omega_a\tau_R < 1$), the characteristic time τ_c increases; in this regime, cells have enough time between oscillations of the applied stress to reorganize their actin stress fibers and focal adhesions to establish the optimal set point in the adjacent matrix. As the frequency of the applied stress further decreases, cells have sufficient time to relax. They are therefore able to balance the internal cellular forces and the matrix forces by orienting parallel to the applied stress direction. Thus, the characteristic relaxation time, τ_c, gradually approaches the relaxation time of the static stress limit (shown by region B). In this regime, where cells orient along the parallel direction, the relaxation time can be estimated by deriving the effective forces in the presence of static stress from the gradient of the total free energy given in Eq. (10), as $\partial f_\theta/\partial\theta \approx 2cp_a(1-2p_a+cp_a)\theta$ in the limit of $\theta \to 0$. For small values of c, the relaxation time can be approximated by, $\tau_c \sim 1/[2cp_a(1-2p_a)]$; for example, for the parameter values of $c=0.01$ and $p_a=0.3$, $\tau_c \sim 416$ and from the exact numerical calculations (shown in Fig. 3) τ_c turns out to also be of the same order ($\sim$360). Similarly, the order of magnitude of τ_c can also be estimated for the other values of c and p_a. In terms of the physical variables, the characteristic relaxation time scales as $\tau_c \sim \tau_R/P_a/E'$ and is mainly governed by the magnitude of the external stress and elasticity of the matrix. Since P_a and E' are known experimentally and τ_R can be measured from relaxation experiments at zero stress (see above), measurement of τ_c provides an important check of the theory.

(iii) Low frequency regime: If the time variation of the applied stress is much slower than the cell's internal relaxation time ($\omega_a\tau_R \ll 1$), the cell can instantaneously follow the external stress and readjust its contractile activity instantaneously to establish the optimal stress in the adjacent matrix. In this regime (shown by region C in Fig. 3), the characteristic relaxation time τ_c is proportional to the time variation of the external stress, i.e., $\tau_c \sim 1/\omega$. Thus, eventually the competition between the cell's internal relaxation time and the

time scale of the external stress sets the cellular response and hence the characteristic relaxation time of the steady-state alignment.

IV. ROLE OF THE MATRIX IN CELL ORIENTATION DYNAMICS

It is well established that the traction forces generated by cells strongly depend on the nature of the substrate and the extra cellular matrix. Numerous experiments show that cells reorganize their cytoskeleton and remodel their contractile forces in a manner that depends on their physical environment. To date, the detailed mechanisms that govern the interaction of cells with the extra cellular matrix and the related biochemical processes during cell spreading, movement or contraction during wound healing are not fully understood. However, some insight into this process can be obtained from the response of a cell to its mechanical environment, since these cell-matrix interactions can trigger biochemical signaling that can then generate a feedback loop [1,2,6,7]. Moreover, a topic of current cell science is how the cell matrix elasticity dictates cell fate. Recent research shows that the matrix elasticity governs the differentiation of stem cells into various cell types, brain, muscle or bone [10]. These findings clearly show that even though the complex ligand-receptor interactions between soluble and matrix molecules are important in regulating cellular function and fate, the mechanical properties of the local microenvironment also can play a key role.

In this section, we predict the dynamics of the cellular orientation as a function of the Poisson ratio of the matrix. For simplicity we take the case of $\alpha=0$ and neglect the self-energy term. In the case of homogeneous isotropic materials, such as most gels, the Young's modulus, E, and the Poisson ratio, ν, completely specify the elastic properties of the material. For a perfectly incompressible material the Poisson ratio, $\nu=1/2$. Most practical engineering materials show values of ν between 0 and 0.5; for example, cork has $\nu\approx 0$, while most steels have $\nu\approx 0.3$, and rubber has $\nu\approx 0.5$ [44,45]. However, some materials, mostly polymer foams, exhibit a negative Poisson's ratio; if these materials are stretched in one direction, they expand in the perpendicular direction, instead of contracting [46,47]. Calculations similar to those presented above show that the steady-state magnitude of the force dipole depends strongly on the Poisson ratio of the matrix but the orientation is not strongly affected.

A. Is cell ativity controlled by stress or strain in the medium?

There are many experimental observations that suggest that cells establish the traction forces to maintain a fixed, tactile set point that depends on the elastic properties of its surrounding matrix [1,13–16]. This regulation is analogous to other physiological set points, e.g., extra-cellular ion concentrations [1]. However, whether cellular activity is controlled by the strain in the medium or by the stress in the medium remains a puzzle so far. In other words, whether it is the force or the deformation of the adjacent matrix, that limits the cellular force is still not clear. For example, measurements by Saez and co-workers [16] of the forces that cells exert on substrates suggest that these traction forces are proportional to the substrate rigidity; in a simple elastic model, this implies that the deformation is constant, suggesting that the cellular forces are regulated by the deformation of the matrix (see Sec. V for a different interpretation). However, the measurements by Freyman *et al.* [15] of the macroscopic contraction of the substrate, shows that the contractile force developed by the cell is limited by the force rather than the displacement of the medium. Here, we suggest that the dependence of cell orientation (in response to high frequency cyclic strain), on the Poisson ratio of the matrix can be used as an experimental probe to distinguish whether it is stress or strain that regulates the cellular activity [35].

B. Strain as the set point

In the model discussed previously we assumed that cells maintain an optimal stress in the adjacent matrix as their tactile set point and in the presence of an external stress, they readjust their internal activity to reestablish the optimal stress condition.

We now consider the possibility that cells readjust their contractile activity by reorganizing the focal adhesions and stress fibers to maintain an optimal strain, $U^{\star}$, in the adjacent matrix instead of an optimal stress, $P^{\star}$. Moreover, we assume that a cell whose axis is along z, regulates its contractile activity in response to the z component of the local reaction strain in the adjacent matrix (following arguments similar to those presented above for the case of stress as tactile set point in Sec. II). The zz component of the local (i.e., located just outside the long edge of the needlelike cell) reaction strain, U_R^c, in the matrix due to a force dipole at the origin, $P_{ij}=P\delta_{iz}\delta_{jz}$, is obtained from Eq. (A7) as

$$U_R^c = -\frac{P(1+\nu)}{a^3\pi E}, \tag{22}$$

where a denotes the cell size, E the Youngs modulus, and ν the Poisson ratio of the matrix. $U_R^c>0$ represents a stretch in the adjacent matrix since the cellular contractile dipole, P, is negative. At this stage, we assume that the cell reorganizes its internal cellular processes so that the local reaction strain, U_R^c, is always maintained at the optimal strain value, $U^{\star}$.

If the matrix is subjected to an external stress, σ_{ij}^a, applied at an angle θ relative to the cell axis (taken to always be along z as illustrated in Fig. 1), then the zz component of the applied strain in the adjacent matrix is [40]

$$U_{zz}^a = (1/E)\{\sigma^a[(1+\nu)\cos^2\theta - \nu]\}. \tag{23}$$

The optimal strain in the matrix (set-point condition) is achieved when the sum of the local reaction strain and the component of the externally applied strain along the cell axis is equal to the optimal strain, i.e., $(U_R^c+U_{zz}^a)=U^{\star}$. Any deviation from this optimal strain condition gives rise to internal cellular forces that will tend to reestablish this set point; those forces are derived from the variation of the effective free energy

$$F_{c,n} = \frac{1}{2}\chi_s(U^a_{zz} + U^c_R - U^\star)^2$$
$$= \frac{\chi_s}{2}\left(\frac{\sigma^a}{E}[(1+\nu)\cos^2\theta - \nu] - \frac{P(1+\nu)}{a^3\pi E} - U^\star\right)^2, \quad (24)$$

where χ_s is the measure of cell activity and has the dimension of energy [48].

In addition to the internal, active cellular forces the cell is also subject to mechanical forces exerted by the matrix. In the presence of an external tensile stress, σ^a_{ij}, the cell-matrix interaction energy, F_m, is given by Eq. (8), derived in Sec. II. Thus, the total generalized force acting on the system can be derived from variations of the total effective free energy, $F = F_{c,n} + F_m$ with respect to changes in both the dipole magnitude and direction. We rewrite the total free energy F in dimensionless units as $f = F/(\chi_s u^{*2})$. In addition, the dimensionless local reaction strain, u_c, due to the dipole, P, is scaled as $u_c = P/(E'U^\star)$, where $E' = E\pi a^3$ is the effective elastic modulus. As before, we define the applied stress magnitude σ^a as $P_a = \sigma^a \pi a^3$ and P_a is then scaled as $u_a = P_a/(E'U^\star)$, where the dimensionless quantity u_a represents the applied strain scaled with respect to the optimal strain, $U^\star$. We also define the dimensionless parameter $c_s = E'/\chi_s$. This 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, $\sigma = \sigma^a(1 - \cos\omega_a t)$, where ω_a is the frequency of the applied stress. To understand the physics more clearly, we first consider the case where active cellular forces are much larger than the matrix forces so that the cell forces control the dynamics of the system. If the time variation, $1/\omega_a$, of the external cyclic stress is much faster than the cellular relaxation time, τ_R, i.e., if $\omega_a \tau_R \gg 1$, then cells cannot instantaneously follow the quickly varying external stress and the long-time solution is obtained by averaging the forces (or equivalently the free energy [48]), over a cycle. The average of the cell energy, $F_{c,n}$, over a cycle is written in dimensionless units as

$$\langle f_{c,n}\rangle = \frac{1}{2}\{u_a[(1+\nu)\cos^2\theta - \nu] - u_c(1+\nu) - 1\}^2$$
$$+ \frac{1}{4}\{u_a[(1+\nu)\cos^2\theta - \nu]\}^2. \quad (25)$$

The second term in Eq. (25) arises from the averaging of the external field over the cycle and adds a positive contribution to the total free energy, compared with the case of static strain. Using this expression, we derive the net, generalized force that acts on the system by calculating the gradient of the effective free energy, $\langle f_{c,n}\rangle$, with respect to θ and u_c. The steady-state value of $u_c = u_c^s$ is obtained by solving the equation $\partial\langle f_{c,n}\rangle/\partial u_c = 0$ and then substituting the value of u_c^s in Eq. (25). We thus obtain the free energy as a function of only the orientation angle, θ, as

$$\langle f_{\theta,n}\rangle = \tfrac{1}{4}\{u_a[(1+\nu)\cos^2\theta - \nu]\}^2. \quad (26)$$

We now solve the equation $\partial\langle f_{\theta,n}\rangle/\partial\theta = 0$, to predict the possible steady-state values of the cellular orientation, θ,

$$\theta^s = 0, \frac{\pi}{2}, \cos^{-1}\sqrt{\frac{\nu}{(1+\nu)}}. \quad (27)$$

For the case of $\nu > 0$, 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 a region of zero strain given by the cosine expression of Eq. (27). This is the physical reason that the cell chooses the zero-strain direction given by Eq. (27) when $\nu > 0$; when there are no strains, the cell is not frustrated by having to adapt its internal activity to the instantaneous, dynamically varying strain at a time scale that is too fast for its natural response. It thus chooses a direction where there is no applied strain and where the cell can establish its contractile machinery in a static manner to achieve the optimal strain, with no dynamically induced frustration. 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. Mathematically, a stability analysis shows that the stable orientation for $\nu < 0$ is $\theta^s = \frac{\pi}{2}$, i.e., the perpendicular direction but for $0 \leqslant \nu \leqslant 0.5$, the steady-state orientation is determined by $\theta^s = \cos^{-1}\sqrt{\frac{\nu}{(1+\nu)}}$ the direction of zero strain.

We note that our prediction of the steady-state cellular orientation, θ^s, in the presence of dynamic stress as a function of ν turns out to be the same as suggested by the authors of Ref. [36]; however, our prediction is based on a model that takes into account both the cell and matrix forces and that can be generalized to treat both the dynamics and steady-state response.

C. Stress as the set point

To treat the case in which the stress determines the cellular set point and the dependence of the cell orientation on the Poisson ratio, we recall our theory of "optimal stress as set point" which was discussed in detail in the preceding sections. At first, we consider the limit of $c \ll 1$ where the cell is governed by the internal cellular forces and neglect the matrix forces. In the presence of a high frequency dynamic stress, we obtain the cellular free energy from Eq. (7) by averaging over a cycle,

$$\langle f_{c,s}\rangle = \tfrac{1}{2}[p_a\cos^2\theta - \beta(\nu)p - 1]^2 + \tfrac{1}{4}p_a^2\cos^4\theta. \quad (28)$$

The steady-state, cellular orientation is predicted by solving $\partial\langle f_{c,s}\rangle/\partial\theta = 0$ and $\partial\langle f_{c,s}\rangle/\partial p = 0$ and the solution is $\theta^s = \frac{\pi}{2}$. This state of perpendicular orientation is the zero stress direction for an applied tensile stress. Since the cell cannot instantaneously follow the quickly varying, external stress, it cannot establish the (time-varying) optimal stress in the adjacent matrix. This frustration effect leads to the fact that for high frequencies, the cell orients in the perpendicular direction to avoid the external stress; in the perpendicular direction there is no time-varying stress and the cell can easily establish its dipole strength to match that of the set point.

In this scenario, where the matrix forces acting on the cell are negligible compared with the internal cellular forces, we

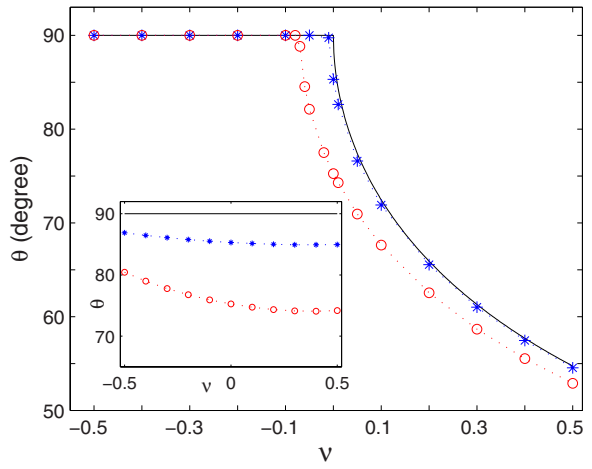

FIG. 4. (Color online) Numerical calculations of the cellular orientation, θ, as a function of the Poisson ratio, ν, of the matrix for the case of strain as the set point for two different values of c_s $=0.001$ ($\star$) and 0.01 ($\bigcirc$) with an applied strain magnitude $u_a=0.3$ and frequency $\omega=10$ using the scaled units discussed in the text. The solid line shows the orientation predicted analytically for the case where the matrix forces are negligible. The small dots are just a guide to the eye. The inset shows a similar plot of θ as a function of ν for the case of stress as set point for $c=0.001$ ($\star$) and 0.01 ($\bigcirc$) with an applied stress magnitude $p_a=0.3$ and scaled frequency ω $=10$. The solid line shows the orientation predicted analytically for the case where the matrix forces are negligible. For values of c (for the same values of p_a) of the order of unity, the cell orients parallel to the direction of the applied stress.

find that in the case of "optimal stress" as the set point, the stable cellular orientation is independent of the Poisson ratio of the matrix, ν. This is in contrast to the case in which the set point is determined by the strain where we found that the steady-state cellular orientation depends on ν as θ_s $=\cos^{-1}\sqrt{\frac{\nu}{1+\nu}}$, as discussed in the preceding section. Thus, measurements of the orientation angle as a function of the Poisson ratio of the matrix differentiate between cells whose set point is governed by optimal stress or by optimal strain.

In Appendix F, we present calculations of the steady-state orientation angle for the cases of both stress and strain as set points and include the effects of the matrix deformation energy. When the deformation forces are small compared with the cell activity forces, i.e., when the parameter c is small, the results are qualitatively similar to the case considered above in which the matrix forces are neglected. Figure 4 presents some representative results that show that for the case of strain as a set point, the steady-state angle for high frequency, dynamic stretch is much more strongly dependent on the Poisson ratio compared with the case of stress as a set point as shown in the inset of Fig. 4. For the cases of strain as set points, we predict a transition in the steady-state θ solution as the Poisson ratio ν varies from negative to positive values. The approximate analytical solution of θ is derived in Eq. (F3) and is given by the inverse cosine of the function $f(\nu,u_a,c_s)$ in Eq. (F2). However, for negative values of ν, where $f(\nu,u_a,c_s)$ becomes imaginary, the stable steady-state solution is then given by $\theta=\pi/2$. The transition in θ solution [from $\pi/2$ to $\cos^{-1} f(\cdots)$] as ν varies from negative to positive values gives rise to the abrupt change in the slope.

V. SUMMARY AND DISCUSSIONS

The comprehensive theory presented here uses an effective free energy that includes terms due to both internal cellular regulation as well as the matrix deformations to predict the forces that determine the dynamics of cellular orientation in the presence of static and cyclically varying external stresses. Our theory predicts many features observed in experimental measurements of cellular forces and orientation: (i) It shows good qualitative agreement with the experimental observation of nearly parallel cellular alignment in the presence of static or quasistatic stress [19–23] and (ii) nearly perpendicular alignment for quickly varying external stresses [24–32]. The competition of the cell activity and the matrix forces determines the steady-state cellular orientation. At low frequency, cells have sufficient time to readjust their contractile activities by reorganizing the cytoskeleton and thus balance the active cell forces by matching their internal forces to optimal set point in the matrix. Since the internal forces are balanced, the cell orientation is then determined by the matrix forces that cause cells to orient parallel to the external stress field. On the other hand, at high frequencies of the applied stress, the cell cannot follow the quickly varying stress to establish its set point; the forces due to the cellular activity thus tend to orient the cell perpendicular to the stress direction so that it remains unaffected by the external stress and can reach homeostasis in the adjacent matrix. Of course, the matrix forces also contribute to the value of the steady-state orientation; for the situation treated here, the matrix forces are relatively small and the cells orient nearly perpendicular.

Our theory also predicts the characteristic relaxation time (the time required to reach to the steady-state orientation) as a function of the frequency of the applied cyclic stress. The relaxation time exhibits three distinct frequency regimes of the applied cyclic stress. Indeed, a recent experiment [42] shows qualitative agreement with our predicted frequency dependence of the characteristic relaxation time for the high frequency regime. We have also identified different scaling regimes of the characteristic relaxation time as a function of the experimental parameters involved in our theory, and these can be used to check the consistency of the theory.

It is important to note here that there are only three theoretical parameters in our model: The cell activity χ, the optimal set point stress $P^\star$, and the cellular relaxation time τ_R. The applied stress, P_a, the matrix elastic modulus, E', and the frequency, ω_a, are experimental variables. One can easily measure the cellular relaxation time and the set-point stress in the absence of stress, by taking cells from solution and setting them in the matrix. Cells slowly spread in the matrix and establish a contractile force that reaches a saturating value $P^\star$ after a time, τ_R [14,19]. Thus, this leaves only one theoretical parameter, the cell activity, χ, to be determined and we suggest several experiments that can be compared with our theory to get a consistent value for χ. First, χ can be found from dynamical stretching experiments by measuring

the steady-state cell orientation angle [Eq. (14), $\cos\theta \sim \sqrt{(P^\star/P_a)/(\chi E')}$]. χ can also be obtained from the characteristic relaxation time measurements [$\tau_c \sim \tau_R/(\chi P_a^2/P^\star)$] in the high frequency regime; from these two measurements, one can check the consistency of our scaling predictions as a function of χ.

Interestingly, in the presence of high frequency cyclic stress, the cell orientation is a nonlinear function of the applied stress, P_a, and we also predict the threshold value of the external stress, $P_a \sim P^\star/(\chi E')$, above which the cell orients towards the perpendicular direction. The existence of a threshold magnitude has also been observed in experiments [42,49,50]. However, there can also be stochastic forces that induce a random alignment of the cells; our analysis can be generalized to include these forces, but the detailed treatment that focuses on the competition of the mechanical and cell-activity forces is outside the scope of this paper.

Furthermore, we predict the variation of the steady-state cell orientation as a function of the Poisson ratio of the matrix for the two possible scenarios of "optimal strain" and "optimal stress" as set points that regulate cell activity. We show that the steady-state cellular orientation has a much stronger dependence on the Poisson ratio of the matrix for the case of optimal strain (for $\nu > 0$) compared with the case of optimal stress, as illustrated in Fig. 4. This difference can be used to distinguish cells whose mechanosensitivity is governed by optimal strain from cells whose activity is controlled by optimal stress. We note however, that the authors of Ref. [51] have suggested that for soft and thick substrates, the size of focal adhesions may be proportional to the elastic modulus of the substrate and that this may explain the results of [16] even if the set point is governed by stress and not by strain.

It is worthwhile to point out that for simplicity, we have presented the numerical results without the self-energy term; however, our analytical stability criteria for cell orientation do include the self-energy term for both static as well as dynamic stresses. We have shown that the self-energy just shifts the numerical values but does not change the qualitative behavior or the scaling regime where the cell activity χ dominates the matrix forces [$c = 1/(\chi E') \ll 1$]. We have also discussed the regularization procedure to calculate the self-energy in Appendix A. In addition, Appendix B shows that the effective free energy that was used to derive the forces acting on the system can also be derived from symmetry considerations. Furthermore, the dynamical equations presented here and derived in Appendix C, are more general than the simplified equations of Refs. [34,35] in which the factor $1/p^2$ that appears in the dynamical equation for the orientation angle was replaced by its steady-state value of approximately unity.

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APPENDIX A: ELASTIC ENERGY OF FORCE DIPOLES

The stress and the strain produced by the force dipole in an isotropic, elastic medium have been described in detail in the literature [37,38,40,52]. Here, for the sake of completeness, we outline these results. The linear, second-order differential elastic equation for the displacement, $\vec{u}$, of an infinite elastic medium at a distance $\vec{r}$ [40] due to a force $\vec{f}$ localized at the origin is

$$\Delta\vec{u} + \frac{1}{1-2\nu}\nabla[\nabla\vec{u}(\vec{r})] = -\frac{\vec{f}(\vec{r})}{\mu}, \tag{A1}$$

where ν and μ are the Poisson ratio and the shear modulus of the elastic medium, respectively. In general, Eq. (A1) is difficult to solve because the second term couples the various components of the displacement vector. However, the equation can be solved by introducing the Green's function for a point force $f_i(\vec{r}) = f_i\delta(\vec{r}-\vec{r}')$. The Green's function for such a force in a three-dimensional, elastic infinite medium is known as the Thomson Green's function [40,52] and is written as

$$G_{ij}(\vec{r}-\vec{r}') = \frac{1+\nu}{8\pi E(1-\nu)|\vec{r}-\vec{r}'|}\left[(3-4\nu)\delta_{ij} + \frac{(r_i-r_i')(r_j-r_j')}{|\vec{r}-\vec{r}'|^2}\right]. \tag{A2}$$

The elastic displacement fields for a more complicated force distribution are obtained from the convolution of the Green's function tensor with the force density (force per unit volume) [36–38,40],

$$u_i(\vec{r}) = \int d\vec{r}' G_{ij}(\vec{r},\vec{r}')f_j(\vec{r}'), \tag{A3}$$

where the subscripts refer to the vector components and the displacement field, $\vec{u}$, at $\vec{r}$ is due to a force distribution density, $\vec{f}$, centered at $\vec{r}'$. The force dipole, P_{ij}, is defined [36–38] as the second moment of a spatially distributed force density, $\vec{f}(\vec{r})$,

$$P_{ij} = \int r_i f_j(\vec{r}) d\vec{r}. \tag{A4}$$

When the force distributions are expanded as force dipoles, the displacement field $u_i(\vec{r})$ at point $\vec{r}$ due to a dipole P'_{kl} at point $\vec{r}'$ is written [38,40,52] as

$$u_i(\vec{r}) = G_{ik,l'}(\vec{r},\vec{r}')P'_{kl}, \tag{A5}$$

where the index after the comma denotes derivative of the Green's function with respect to position. Moreover, we can find the components of the strain tensor, $u_{ij}(\vec{r})$, from the displacement using the relation

$$u_{ij} = \frac{1}{2}\left(\frac{\partial u_i}{\partial r_j} + \frac{\partial u_j}{\partial r_i}\right). \tag{A6}$$

Thus, the strain tensor is given by Eqs. (A5) and (A6),

$$u_{ij}(\vec{r}) = \tfrac{1}{2}[G_{ik,l'j}(\vec{r},\vec{r}') + G_{jk,l'i}(\vec{r},\vec{r}')]P'_{kl}, \tag{A7}$$

where

$$G_{ik,l'j} = \frac{\partial}{\partial x_j}\frac{\partial}{\partial x'_l}G_{ik}(\vec{r}-\vec{r}') \tag{A8}$$

and there is an implied summation over repeated indices. Note that for translationally invariant systems, $G_{ij,lk'}(\vec{r},\vec{r}') = G_{ij,lk'}(\vec{r}-\vec{r}') = -G_{ij,lk}(\vec{r},\vec{r}')$ [38]. We note that the symmetric definition of the strain as a sum over two Green's functions in Eq. (A7) corrects the general formulas presented in [37,38]. The symmetrization also gives rise to quantitative changes in the Poisson ratio dependence of the self-energy.

1. Interaction of dipoles with external stress

In the absence of any external forces, the elastic deformation energy [40] of an infinite medium due to a single force dipole is derived from the strain field using Eq. (A7). This energy is also termed as the self-energy of the dipole and is given by

$$U_S = \frac{1}{2}\int C_{ijkl}u_{ij}(\vec{r})u_{kl}(\vec{r})d^3r = \frac{1}{2}\int \sigma_{ij}(\vec{r})u_{ij}(\vec{r})d^3r = \frac{P^2}{2E\pi a^3}\alpha(\nu), \tag{A9}$$

where E is the Young's modulus, ν is the Poisson ratio of the matrix. The Poisson ratio dependent function $\alpha(\nu)$ and its precise form depends on the regularization procedures to eliminate short distance singularities and is discussed in the next section [53].

We now derive the interaction energy of the force dipole with an external applied strain. This is analogous to the interaction of an electric dipole with an external electric field. We focus on the dilute limit, in which the depolarization fields [54] due to all other dipoles in the system are negligible; this may not be the case for systems with a high density of cells. For an applied tensile stress, σ^a_{ij}, the total strain in the medium is $u^T_{ij} = (u^c_{ij} + u^a_{ij})$, where u^c_{ij} is the strain due to the force dipole and u^a_{ij} is the strain due to the external stress. The total elastic deformation energy can be calculated by considering the work to increase the total strain in the medium from zero to a value of u^T_{ij} [37],

$$F_m = \frac{1}{2}\int C_{ijkl}u^T_{ij}(\vec{r})u^T_{kl}(\vec{r})d^3r - \frac{1}{2}\int C_{ijkl}u^a_{ij}(\vec{r})u^a_{kl}(\vec{r})d^3r. \tag{A10}$$

The second term in Eq. (A10) represents the work done by the external force to maintain the applied stretch, u^a_{ij}; it is not relevant to the forces that act on the cell and does not couple to the cellular strain [37]. We thus subtract this term in our accounting of the effective energies that give rise to forces that act on the cell.

Thus, the relevant, elastic deformation energy in the presence of an external strain is

$$F_m = \frac{1}{2}\int C_{ijkl}u^c_{ij}(\vec{r})u^c_{kl}(\vec{r})d^3r + \int C_{ijkl}u^c_{ij}(\vec{r})u^a_{kl}(\vec{r})d^3r = U_S + U_I. \tag{A11}$$

The first term in Eq. (A11) represents the self-energy, U_S, of the force dipole [as discussed in Eq. (A9)] and the second term represents the interaction energy, U_I, of the force dipole with an external, tensile strain, u^a_{ij}. Now, integrating the interaction energy term by parts yields

$$U_I = \int u^c_{ij}C_{ijkl}u^a_{kl}d^3r = \int ds_j u^a_i C_{ijkl}\partial_l u^c_k - \int u^a_i C_{ijkl}\partial_j\partial_l u^c_k d^3r, \tag{A12}$$

where the first term is a surface integral. In the presence of an external stress, σ^a_{ij}, the boundary condition yields $C_{ijkl}(u^a_{kl}+u^c_{kl})n_j(\vec{r}) = \sigma^a_{ij}n_j(\vec{r})$, where $\vec{n}$ is the outward normal of the surface. We define the uniform strain, u^a_{ij}, due to the applied stress from, $\sigma^a_{ij}n_j(\vec{r}) = C_{ijkl}u^a_{kl}n_j(\vec{r})$. For a dilute system, the surface forces due to dipoles are negligible, so that $C_{ijkl}u^c_{kl}n_j(\vec{r}) = 0$. This yields a vanishing surface integral [first term of Eq. (A12)] at the boundary. Moreover, mechanical equilibrium ensures that the body force due to the dipole, $f_i(\vec{r})$, is balanced by the internal restoring force, $\sigma_{ij,j}$. This leads to the mechanical equilibrium condition, $\sigma_{ij,j} = C_{ijkl}\partial_j\partial_l u^c_k = -f_i(\vec{r})$. Setting this expression in the second term of Eq. (A12) we find

$$U_I = \int u^a_i f_i d^3r. \tag{A13}$$

For a constant applied stress, the displacement, $u^a_i = r_j u^a_{ij}$, where u^a_{ij} is the constant strain. Using the definition of the force dipole, Eq. (A4), we thus find

$$U_I = P_{ij}u^a_{ij}. \tag{A14}$$

The interaction energy is, thus, proportional to the product of the force dipole and the external strain. This adds a negative energy contribution (since $P<0$ and $u^a_{ij}>0$) to the total energy that is minimal when the dipoles are aligned parallel to the stretch direction. The physical origin of the mechanical forces that tend to align the force dipoles parallel to the external strain is the fact that the external stretch expands the matrix, while cells that are parallel to the strain contract the matrix, thus reducing the overall strain and lowering the matrix deformation energy.

2. Self-energy

The self-energy of a dipole located at the origin of an infinite, elastic medium is given by Eq. (A9). Since the strains due to a dipole at the origin vary as $1/r^3$, the self-energy, which is an integral of the square of the strain multiplied by the appropriate elastic constant, diverges [53]. One must regularize this divergence by a short distance cutoff. In our previous work [34,35], we removed the divergence by assuming that the cell occupied a finite volume near the origin and that the strained medium was excluded from this volume. A technical aspect of this regularization is corrected

below. In addition, other regularization schemes that do not require the construction of a "hole" in the medium are discussed.

The technical correction to [34,35] is due to the fact that those papers did not use the properly symmetrized form of the strain in Eq. (A7). Only the first term (without the factor of $1/2$) was used. This makes no difference for the diagonal components of the strain tensor that enter the cell activity energy and for the interaction of the force dipole with the external force. However, the off-diagonal components of the strain tensor that enter the self-energy are modified by the symmetrization. This results in the fact that the Poisson ratio-dependent prefactor, $\alpha(\nu)$ is modified from the value given in the previous work [34,35],

$$\alpha(\nu) = (1+\nu)[15+2\nu(-13+8\nu)]/[15(\nu-1)^2]. \tag{A15}$$

In that work, the integrals were performed in spherical coordinates; since the strain varies as $1/r^3$, we introduced a short-distance cutoff, related to the cell size, a, which was taken to be $a/(2^{1/3})$. The correct value of $\alpha(\nu)$ in the properly symmetrized model is

$$\alpha(\nu) = (1+\nu)[9+2\nu(-7+5\nu)]/[30(\nu-1)^2], \tag{A16}$$

where, the integration cutoff is taken to be a. This, of course, makes no qualitative difference in the predictions of cell orientation.

We begin by noting that we consider a force dipole associated with a needlelike cell whose focal adhesions and stress fibers are aligned along z, so the dipole has only zz components. However, to derive the self-energy, the integrals were performed in spherical coordinates with a short-distance cutoff, related to the cell size, a. However, this approximation corresponds to a picture in which the cell creates a "hole" in the matrix which introduces a boundary at $r \sim a$. To perform the integration over an infinite medium, boundary forces may have to be taken into account and this may require more detailed modeling of the cellular forces.

In addition, we note that to get a more accurate expression for the dependence of $\alpha(\nu)$ in the self-energy, one should probably integrate over a needlelike geometry rather than a sphere. This would be more consistent with our treatment of an elongated cell whose only force dipole component is P_{zz}. Moreover, there are many possible regularization schemes that can be used to treat the short distance cutoff (e.g., sharp cutoff in wave-vector space [53], Gaussian force dipole distribution, etc.). The precise value of $\alpha(\nu)$ may depend on the details of these procedures and their relation to the cell shape.

However, these details are not crucial for our predictions of cell orientation. First, the self-energy does not couple to the orientation: It is 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. Even if the mechanical forces were larger than the cell-activity forces, the self-energy only influences the physics indirectly, via the magnitude of the force dipole. Moreover, because cells polarize nearly perpendicular to the direction of rapidly varying stresses, the mechanical forces (that tend to align the cell parallel to the external stress) must be secondary compared with the cell-activity forces. This paper thus considers the limit in which the cell-activity forces dominates the matrix elastic force. We therefore neglect the self-energy term in the numerical calculations of cell orientation used in the paper. We note that, in our theory, the interaction energy of the force dipole with the external applied stress (that linearly couples the external stress and the dipole) is crucial in determining cell orientation for the case of applied static stress. The competition of this interaction energy term with the cell energy eventually determines whether parallel or perpendicular cellular alignment is the minimum energy state.

APPENDIX B: FREE ENERGY FROM SYMMETRY CONSIDERATIONS

The free energy associated with a force dipole in the presence of an external stress can be written in a phenomenological manner using symmetry considerations. Here we consider the special case of a needlelike cell whose long axis is along the $\vec{r}$ direction and at an angle θ relative to the z axis of the matrix. In the main body of the text we have interchanged the coordinate system of the cell and the matrix but that does not affect the form of the free energy. We also limit ourselves to the special case in which the stress fibers are all oriented along the long axis of the cell. Thus, the only nonvanishing element of the force-dipole tensor associated with the cell, is (in spherical coordinates where the tensor elements are denoted by rr, $r\theta$, $r\phi$, θr, $\theta\theta$, etc.), the one associated with the tensor index, rr. We can also represent this tensor by the two vectors for the cell axis and the force, which for our special case are $\vec{r}_0=(r_0,\theta,\phi)$ and $\vec{f}_0=(f_0,\theta,\phi)$.

Since the force dipole is composed of two equal and opposite forces, $\vec{f}_0$ and $-\vec{f}_0$, located at the two opposite ends of the needlelike cell, $\vec{r}_0$ and $-\vec{r}_0$, the free energy must be an even function of the product of $\vec{f}_0$ and $\vec{r}_0$. Thus, as long as we enforce this symmetry, we can consider the two vectors, $\vec{f}_0$ and $\vec{r}_0$ instead of the force dipole tensor that is composed of these two vectors.

The stress tensor, $\mathbf{T}$, for a uniaxial stretch applied in the $\hat{z}$ direction and perpendicular to the surface of the matrix whose normal is in the $\hat{z}$ direction is a 3×3 matrix (with elements xx, xy, xz, yx, yy, etc.) whose only nonvanishing component is zz. We denote the magnitude of this component by T. It is equal to the trace of the stress tensor, which is of course a scalar.

We now write the vectors $\vec{r}_0$ and $\vec{f}_0$ in rectangular coordinates using the usual transformation from spherical to rectangular geometry and then form the scalar invariants of $\vec{r}_0, \vec{f}_0$ and the stress tensor as described above. The vector that corresponds to the long axis of the cell is written in rectangular coordinates as

$$\vec{r}_0 = r_0(\cos\phi\sin\theta, \sin\phi\sin\theta, \cos\theta). \tag{B1}$$

The equation for the force vector is similar with r_0 replaced by f_0. We note that in our model of needlelike cells, $\vec{f}_0$ and $\vec{r}_0$

are parallel so that their cross product is zero. Assuming that both the force and the stress are small, we write the free energy up to quadratic order (in either quantity, but not in both) in terms of the scalar invariants,

$$F_p = a_0 + a_1 \vec{r}_0 \cdot \vec{f}_0 + a_2 \vec{r}_0 \cdot \mathbf{T} \cdot \vec{f}_0 + a_3 \vec{r}_0 \cdot \mathbf{T} \cdot \vec{r}_0 \quad \text{(B2)}$$

$$+ a_4 (\vec{r}_0 \cdot \vec{f}_0)^2 + a_5 \vec{f}_0 \cdot \vec{f}_0 + a_6 (\vec{r}_0 \cdot \mathbf{T} \cdot \vec{r}_0)^2 + a_7 T (\vec{r}_0 \cdot \vec{f}_0), \quad \text{(B3)}$$

where the last term is the trace of the tensor **T** multiplied by the scalar $(\vec{r}_0 \cdot \vec{f}_0)$ There are two additional terms that are proportional to the trace and the square of the trace of the stress tensor. However, these two terms are not coupled to the cell shape or force and can therefore be omitted. For the case we consider, the other invariants—the determinant and the sum of the principal minors—vanish.

The symmetry based, phenomenological free energy, F_p, has the same form as the free energy written in the text [Eq. (9)] from more physical arguments that include the cell regulation of the force dipole and the matrix elastic energies. Note that in both treatments, the stress enters with the factor $\cos^2 \theta$.

APPENDIX C: GENERAL RELAXATION DYNAMICAL EQUATIONS

Symmetry considerations can also be used to derive a relationship between the dynamics of the various components of the cellular stress. For simplicity, we assume that the cell shape and size is fixed, so that the magnitude of $\vec{r}_0$ is constant. For our model of a needle-shaped cell, $\vec{r}_0$ is parallel to the force $\vec{f}_0$ so that we need only determine the angle of $\vec{f}_0$. We write a relaxational equation for the force $\vec{f}_0$, that assumes, for simplicity, a single time scale; the time derivative of $\vec{f}_0$ is proportional to the variation of the free energy with respect to $\vec{f}_0$,

$$\frac{\partial \vec{f}_0}{\partial t} = -\frac{1}{\tilde{\tau}_0} \frac{\partial F}{\partial \vec{f}_0}. \quad \text{(C1)}$$

Our assumption of a single time scale means that in rectangular coordinates, $\partial f_{0,i}/\partial t$ is not coupled to $\partial F/\partial f_{0,j}$ unless $j=i$.

Writing the dynamical equation in spherical coordinates for the case described above where $\vec{f}$ is parallel to the $\hat{r}_0$ direction, we find

$$\frac{\partial \vec{f}_0}{\partial t} = \frac{\partial (f_0 \hat{r}_0)}{\partial t} = \hat{r}_0 \frac{\partial f_0}{\partial t} + f_0 \frac{\partial \hat{r}_0}{\partial t}. \quad \text{(C2)}$$

The last term on the right-hand side can be written as

$$f_0 \frac{\partial \hat{r}_0}{\partial t} = f_0 \hat{\phi} \sin\theta \frac{\partial \phi}{\partial t} + f_0 \hat{\theta} \frac{\partial \theta}{\partial t}. \quad \text{(C3)}$$

The term $\frac{\partial F}{\partial \vec{f}_0}$ can be written in terms of the gradient in spherical coordinates,

$$\frac{\partial F}{\partial \vec{f}_0} = \hat{r}_0 \frac{\partial F}{\partial f_0} + \hat{\theta} \frac{1}{f_0} \frac{\partial f}{\partial \theta} + \hat{\phi} \frac{1}{f_0 \sin\theta} \frac{\partial F}{\partial \phi}. \quad \text{(C4)}$$

Since the free energy is independent of ϕ, there are no gradients of F in the $\hat{\phi}$ direction. Thus, $\frac{\partial \phi}{\partial t} = 0$. Equating the vector components of the terms involving the time derivatives and the terms involving the derivatives of the free energy yields

$$\frac{\partial f_0}{\partial t} = -\frac{1}{\tilde{\tau}_0} \frac{\partial F}{\partial f_0},$$

$$\frac{\partial \theta}{\partial t} = -\frac{1}{\tilde{\tau}_0} \frac{1}{f_0^2} \frac{\partial F}{\partial \theta}. \quad \text{(C5)}$$

Moreover, we have assumed that the cell shape and size is fixed, so that r_0 is constant. Thus, the relaxation equations of the dipole magnitude, $p(=f_0 r_0)$ and the cell orientation, θ, is given as

$$\frac{\partial P}{\partial t} = -\frac{1}{\tau_0} \frac{\partial F}{\partial P},$$

$$\frac{\partial \theta}{\partial t} = -\frac{1}{\tau_0} \frac{1}{P^2} \frac{\partial F}{\partial \theta}, \quad \text{(C6)}$$

where $\tau_0 = r_0^2/\tilde{\tau}_0$. Here F and P have dimensions of energy and t is the time; thus, the dimension of the rate constant τ_0 turns out to be time devided by energy. We, therefore, define a scaled relaxation time $\tau_R = \tau_0 P^\star$ which has the dimensions of time.

We note here that the dynamical equations that determine the relaxation of the magnitude of the force dipole, P, and its orientation, θ, given in Refs. [34,35] were expressed in a simplified form under the assumption that P reaches its equilibrium value $\sim P^\star$ (in scaled units $p \sim 1$) much faster than the cell orientation angle θ. This amounts to replacing the factor $1/P^2$ in Eq. (C6) by $1/(P^\star)^2$ or equivalently by redefining the relaxation time of cell orientation θ as $\tau_\theta \sim [\tau_R (P^\star)^2]$ as given in Refs. [34,35]. For static stresses, the steady-state solution is independent of the details of the dynamical equations and both treatments yield identical results. However, even for time-dependent stresses, the steady-state solutions are qualitatively similar and do not depend in an important manner on whether one replaces the factor of $1/P^2$ in Eq. (C6) by $1/(P^\star)^2$. In fact, this condition is always satisfied in case of high frequency cyclic stresses as well as for cases in which the magnitude P_a, of the external applied stress is small enough so that P reaches $\sim P^\star$ in the steady state. To highlight this, we compare in Fig. 5 the cellular orientation, θ, calculated from the simplified dynamical equations in Refs. [34,35] as well as from the more general dynamical equation given in Eq. (15) as a function of frequency ω for two magnitudes $p_a = 0.2$ and 0.4 of the applied stress (in the scaled units discussed in Sec. II). As seen from the figure, the steady-state values of θ remain the same for the high frequency regime and its qualitative nature is also unaffected for the low frequency regime. For very small values of c and p_a, there is virtually no difference between the

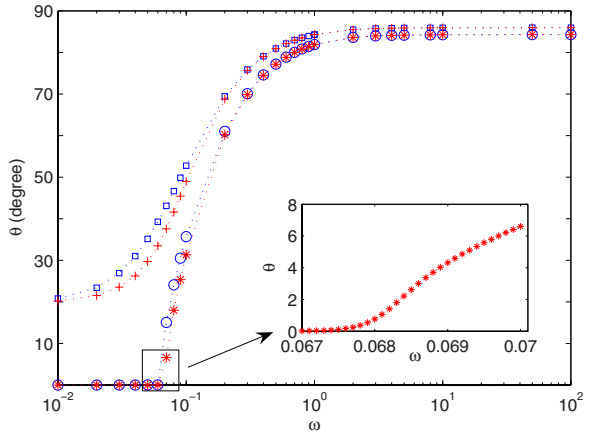

FIG. 5. (Color online) This plot shows a comparison of the dynamical solution of the cell orientation θ as a function of frequency $\omega(=\omega_a\tau_R)$ for the simplified [in which the factor of $1/p^2$ in Eq. (15) is replaced by unity] and general relaxation equation [Eq. (15)] for two different values of the applied stress magnitude $p_a=0.2$ (shown by the symbols ○ and ★) and $p_a=0.4$ (shown by the square and plus symbols) keeping $c=0.001$. The deviations between the two theories can be seen for large applied stress magnitude p_a. (The dotted lines are just a guide to the eye.) The inset focuses on the steady-state solutions of θ as a function of ω for the regime highlighted in the square box. It shows that θ changes smoothly with ω and there is no sharp transition.

simplified and more general dynamical equations. For low frequencies, cells orient towards the parallel direction; as the frequency increases cells orient towards the perpendicular direction. This occurs smoothly as the frequency is varied from low to high values as seen from the plot of the orientation for large values of p_a (=0.4). Also, for smaller values of p_a (=0.2), θ changes smoothly with ω and there is no sharp transition as illustrated in the inset.

APPENDIX D: DYNAMICAL AVERAGE OF TOTAL FORCE OVER ONE CYCLE TO PREDICT LONG-TIME SOLUTIONS

The dynamical behavior of the system yields a linear relation between the temporal change of the dipole magnitude, p, and the orientation angle, θ, and the forces that are derived from the variation of the effective free energy, f (for the case of zero Poisson's ratio),

$$f=\tfrac{1}{2}(p_a\cos^2\theta-p-1)^2+\tfrac{1}{2}c\alpha p^2+cpp_a\cos^2\theta,$$

$$\frac{\partial f}{\partial p}=-(p_a\cos^2\theta-p-1)+c\alpha p+cp_a\cos^2\theta,$$

$$\frac{\partial f}{\partial\theta}=[-p_a(p_a\cos^2\theta-p-1)-cpp_a]\sin 2\theta. \qquad \text{(D1)}$$

However, in the presence of a rapidly varying cyclic external stress, $\sigma^a(1-\cos\omega_a t)$, for the case that time variation, $1/\omega_a$, of the applied stress is short compared with the internal cellular relaxation time, τ_R, the long-time solution is estimated by averaging the effective forces over a cycle,

$$\left\langle\frac{\partial f}{\partial p}\right\rangle=\frac{\omega}{2\pi}\int_0^{2\pi/\omega}\left(\frac{\partial f}{\partial p}\right)dt,$$

$$\left\langle\frac{\partial f}{\partial\theta}\right\rangle=\frac{\omega}{2\pi}\int_0^{2\pi/\omega}\left(\frac{\partial f}{\partial\theta}\right)dt. \qquad \text{(D2)}$$

The steady-state solutions are then given by the solutions of the coupled equations $\langle\frac{\partial f}{\partial p}\rangle=0$ and $\langle\frac{\partial f}{\partial\theta}\rangle=0$ as $\theta_s\to 0,\pi/2$, $\cos^{-1}\sqrt{\frac{2c(1+\alpha)}{p_a\{1+c[(4+3\alpha)-2c]\}}}$. This shows that the derivation of the steady-state orientations obtained by averaging the force over a cycle is equivalent to that obtained from averaging the effective free energy over a cycle.

APPENDIX E: ANALYTICAL ASYMPTOTIC SOLUTION: CALCULATION OF FOURIER COEFFICIENTS

The dynamical relaxation equations for the variables p and ϕ are (see Sec. III D for detailed discussions)

$$g_p(p,\phi,\tau)=\frac{dp}{dt}+\frac{1}{\tau_R}\left(\frac{\partial f_\phi}{\partial p}\right),$$

$$g_\phi(p,\phi,\tau)=\frac{d\phi}{dt}+\frac{1}{\tau_R p^2}[4\phi(1-\phi)]\left(\frac{\partial f_\phi}{\partial\phi}\right), \qquad \text{(E1)}$$

where $f_\phi=\frac{1}{2}(p_a\phi-p-1)^2+cp_apq+\frac{1}{2}c\alpha p^2$ is the total effective free energy. For high frequency applied cyclic stress, the solution can be assumed as the sum of a sinusoidal oscillation at the fundamental frequency ω described as (discussed in Sec. V in detail)

$$p(\tau)=a_0+a_1\sin\omega\tau+a_2\cos\omega\tau,$$

$$\phi(\tau)=b_0+b_1\sin\omega\tau+b_2\cos\omega\tau. \qquad \text{(E2)}$$

The Fourier coefficients a_0,a_1,a_2 and b_0,b_1,b_2 can be found by solving six equations that are obtained by substituting the solutions $p(\tau)$ and $\phi(\tau)$ in Eq. (E1) and then integrating these equations over one period (to $2\pi/\omega$) after multiplying by either unity, $\sin\omega\tau$ or $\cos\omega\tau$ appropriately. Thus, the six algebraic equations yield

$$\int_0^{2\pi/\omega}g_p(p,\phi,\tau)d\tau=0,$$

$$\int_0^{2\pi/\omega}g_p(p,\phi,\tau)\sin\omega\tau d\tau=0,$$

$$\int_0^{2\pi/\omega}g_p(p,\phi,\tau)\cos\omega\tau d\tau=0,$$

$$\int_0^{2\pi/\omega}g_\phi(p,\phi,\tau)d\tau=0,$$

$$\int_0^{2\pi/\omega}g_\phi(p,\phi,\tau)\sin\omega\tau d\tau=0,$$

$$\int_0^{2\pi/\omega} g_\phi(p,\phi,\tau)\cos\omega\tau d\tau = 0. \quad \text{(E3)}$$

In the high frequency regime of the applied cyclic stress, the first Fourier mode is a good approximation for the dynamics and therefore the higher harmonics are ignored in obtaining the solutions. Moreover, the Fourier coefficients are expanded in a power series of $(1/\omega)$ (with the appropriate symmetry) as

$$a_0 = a_{00} + \frac{a_{02}}{\omega^2}, \quad a_1 = \frac{a_{11}}{\omega} + \frac{a_{13}}{\omega^3}, \quad a_2 = \frac{a_{22}}{\omega^2} + \frac{a_{24}}{\omega^4}, \quad \text{(E4)}$$

and similarly

$$b_0 = b_{00} + \frac{b_{02}}{\omega^2}, \quad b_1 = \frac{b_{11}}{\omega} + \frac{b_{13}}{\omega^3}, \quad b_2 = \frac{b_{22}}{\omega^2} + \frac{b_{24}}{\omega^4}. \quad \text{(E5)}$$

The equations for a_{00} and b_{00} are nonlinear. For the other Fourier coefficients, they are found in the high frequency limit, by solving the linearized equations (E3) and equating the coefficients of the equal powers of $\frac{1}{\omega}$. In the high frequency regime, the steady-state solutions of the force dipole, p, and the orientation, θ, can be approximated by the constant Fourier coefficients a_0 and b_0, respectively.

APPENDIX F: COMPETITION OF MATRIX FORCES WITH THE CELLULAR FORCES FOR THE CASES OF BOTH STRAIN AND STRESS AS SET POINTS

In Sec. IV, we have predicted the orientation of cells as governed by the cellular forces alone. In this appendix, we derive the steady-state orientation including the effects of the mechanical forces that are due to the matrix elasticity [derived from Eq. (8) in scaled units for $\alpha=0$]. In this case, one derives the total force acting on the system from the variation of the total effective free energy. We first examine the case of "optimal strain" and show how the cellular and mechanical forces compete to determine the steady-state value of the cell orientation. For high frequency, external dynamic stress, i.e., when $\omega_a \tau_R \gg 1$, we predict the long-time solution by averaging the total effective free energy over a cycle, to yields

$$\langle f_n \rangle = \tfrac{1}{2}\{u_a[(1+\nu)\cos^2\theta - \nu] - u_c(1+\nu) - 1\}^2 + \tfrac{1}{4}\{u_a[(1+\nu)\cos^2\theta - \nu]\}^2 + c_s u_a u_c[(1+\nu)\cos^2\theta - \nu]. \quad \text{(F1)}$$

The second term $\{\frac{1}{4}u_a^2[(1+\nu)\cos^2\theta - \nu]^2\}$ of Eq. (F1) adds a positive contribution to the effective free energy that vanishes for perpendicular alignment and is maximal for parallel alignment. This term tends to drive the system towards perpendicular alignment compared with the matrix forces that drive the cell to the parallel orientation. Therefore, the equilibrium cellular orientation is determined by the competition of these two forces. If the strength of the applied external strain, u_a, is large enough compared with c_s (increasing c_s represents an increase in matrix forces) the system is driven towards the perpendicular direction. Thus, the ratio (c_s/u_a) determines the steady-state cellular orientation (for fixed values of ν).

The steady-state solution is obtained by solving the coupled equations $\partial\langle f_n\rangle/\partial\theta = 0$ and $\partial\langle f_n\rangle/\partial p = 0$. These predict the possible cellular orientations as

$$\theta = 0, \frac{\pi}{2}, \cos^{-1} f(\nu, u_a, c_s), \quad \text{(F2)}$$

where $f(\nu, u_a, c_s)$ is a function of ν, u_a, and c_s that can be approximated as

$$f \sim \sqrt{\frac{\nu}{(1+\nu)}} + \frac{c_s}{u_a}\frac{1}{(1+\nu)\sqrt{\nu(1+\nu)}} \quad \text{(F3)}$$

in the limit of $(c_s/u_a) \ll 1$. A stability analysis shows that the stable orientation is given by the cosine solution when $(c_s/u_a) \ll 1$. However, if the applied field is too small, the driving force for the perpendicular cellular orientation [arising from the second term of Eq. (F1)] is negligible and the stable cellular orientation is $\theta^s = 0$, i.e., along the parallel direction. Indeed, there exists a minimum value of the applied stress above which cells orient in the perpendicular direction.

We have also predicted the dynamics of cellular orientation by solving the coupled relaxation equations,

$$\frac{du_c}{dt} = -\frac{1}{\tau_R}\frac{\partial f}{\partial u_c}, \quad \frac{d\theta}{dt} = -\frac{1}{\tau_R u_c^2}\frac{\partial f}{\partial \theta}, \quad \text{(F4)}$$

where $f = f_{c,n} + f_m$, the total free energy in scaled units. For our numerical calculations, we define the dimensionless scaled time $\tau = t/\tau_R$ and dimensionless frequency of the applied stress as $\omega = \omega_a \tau_R$. Figure 4 shows the cellular orientation, θ, at steady state as a function of the Poisson ratio, ν. We show the solution for two different values of $c_s = 0.001$ (★) and 0.01 (○) for scaled applied strain $u_a = 0.3$; an increase in c_s represents an increase in the matrix forces relative to the cellular forces. For values of c_s close to unity or greater, for fixed applied stress (or equivalently, fixed u_a), the cell orientation is parallel to the applied stress. Comparing the steady-state orientation determined by the cellular forces alone (solid line in Fig. 4) with the orientation due to both the cellular and matrix forces, we find that for positive ν values, an increase in matrix forces causes the steady-state orientation angle to decrease (become more parallel). This happens because the matrix forces drive the cell towards parallel orientation so that the cell contractility can balance the external stretch (and thus at least partially restore the matrix to its uncompressed state); the competition between the cellular and matrix forces eventually sets the steady-state orientation.

We next consider the competition between the cellular and matrix forces for the case where the set point is controlled by an "optimal stress" in the matrix. In this case, the average over a cycle of the total free energy of Eq. (9) (considering $\alpha=0$) is

$$\langle f^s \rangle = \tfrac{1}{2}[p_a \cos^2\theta - \beta(\nu)p - 1]^2 + \tfrac{1}{4}(p_a\cos^2\theta)^2 + cpp_a[(1+\nu)\cos^2\theta - \nu]. \quad \text{(F5)}$$

The steady-state cellular orientation is then θ^s

$=0,\pi/2,\cos^{-1} g(\nu,p_a,c)$, where the function $g(\nu,p_a,c)$ is a complicated function of ν, p_a, and c which can be approximated as

$$g \sim \frac{1}{\beta}\sqrt{\frac{2c}{p_a}}\sqrt{\beta[1+\nu(1+p_a)]} \tag{F6}$$

for $(c/p_a) \ll 1$. The numerical results for the steady-state orientation are plotted in the inset of Fig. 4 for two 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 ratio compared with the case in which the strain is the set point. For large values of the matrix forces, i.e., for values of c (for fixed p_a) of the order of unity, cells orient parallel to the direction of applied stress.

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