Active self-polarization of contractile cells in asymmetrically shaped domains

A. Zemel and S. A. Safran
Department of Materials and Interfaces, The Weizmann Institute of Science, Rehovot 76100, Israel
(Received 5 January 2007; published 7 August 2007)

Mechanical forces generated by contractile cells allow the cells to sense their environment and to interact with other cells. By locally pulling on their environment, cells can sense and respond to mechanical features such as the local stress (or strain), the shape of a cellular domain, and the surrounding rigidity; at the same time, they also modify the mechanical state of the system. This creates a mechanical feedback loop that can result in self-polarization of cells. In this paper, we present a quantitative mechanical model that predicts the self-polarization of cells in spheroidally shaped domains, comprising contractile cells and an elastic matrix, that are embedded in a three-dimensional, cell-free gel. The theory is based on a generalization of the known results for passive inclusions in solids to include the effects of cell activity. We use the active cellular susceptibility tensor presented by Zemel *et al.* [Phys. Rev. Lett. **97**, 128103 (2006)] to calculate the polarization response and hence the elastic stress field developed by the cells in the cellular domain. The cell polarization is analyzed as a function of the shape and the elastic moduli of the cellular domain compared with the cell-free surrounding material. Consistent with experiment, our theory predicts the development of a stronger contractile force for cells in a gel that is surrounded by a large, cell-free material whose elastic modulus is stiffer than that of the gel that contains the cells. This provides a quantitative explanation of the differences in the development of cellular forces as observed in free and fixed gels. In the case of an asymmetrically shaped (spheroidal) domain of cells, we show that the anisotropic elastic field within the domain leads to a spontaneous self-polarization of the cells along the long axis of the domain.

DOI: 10.1103/PhysRevE.76.021905 PACS number(s): 87.15.La, 87.17.Aa, 87.18.La

I. INTRODUCTION

Mechanical forces provide an important route for communication among cells, and between cells and their environment [1–3]. Many cell types, including muscle cells, fibroblasts, endothelial cells, and others, generate significant contractile forces as part of their physiological function. These forces are also thought to serve a more general purpose in living cells, since they provide the cells with a means to sense the elastic nature of their environment [1–5]. Purely mechanical features, such as the rigidity of the medium in which the cells are found, and the existence of elastic stresses (or strains), have been shown to affect important cellular processes, such as changes in cell shape and alignment, cell division, and even differentiation and apoptosis [1–3,6]. Because the propagation of elastic forces within tissues can be long ranged, the local stress field sensed by the cells also reflects global mechanical characteristics of the tissue, such as its shape and its relative stiffness compared to its surroundings. The importance of these physical effects to tissue morphogenesis, cancer development, and tissue engineering has been pointed out by others [7–12]. In the theory presented below, we show that changes in the active cell contractility in response to the local elastic stress can lead to a spontaneous cellular alignment [13] in anisotropically shaped domains of cells.

The contractile force in cells is generated by the actin-myosin stress fibers that generally connect opposite sides of a cell and terminate at specific protein complexes, called focal adhesions, that anchor the cell to its surroundings [14]. In many types of contractile cells, mechanical forces were found to induce an active reorganization of the cellular cytoskeleton, including the focal adhesions and stress fibers, thus leading to changes in the forces (in both magnitude and direction) exerted by the cells on the surrounding medium [15,16]. For static or quasistatic strain (on the scale of tens of minutes), cells generally align parallel to the direction of principal strain [17–19], while for cyclic strain, cells align away from the direction of the applied stretch [6,20–22]. The elastic field that influences the cell polarization can either be applied externally or be generated internally by the contractile forces due to the cells. In this latter case, the cell polarization changes in response to the stresses in the surroundings that originate in the forces produced by the cells themselves. We therefore term this effect self-polarization. This response occurs at relatively long times (typically tens of minutes [12]), after the cells have had enough time to reorganize their stress fibers and adhesions in response to stress; it is therefore relevant for the cellular response to a static or quasistatic field rather than to a rapidly varying (e.g., on the scale of hertz) field. This is also consistent with the experiments we cite below.

Self-polarization can be pronounced when the surrounding matrix is rigid and completely opposes the contractile forces generated by the cells [3]. The clearest demonstration of this behavior is the difference between the behavior of cells in fixed and floating gels [23]. Experiments on fibroblasts in a fixed gel with cylindrical symmetry showed that, while the cells developed pronounced stress fibers in random directions, the magnitude of their contractility was larger than that of cells placed in a freely floating gel in solution [23]. In this case, the reorganization of the cellular force pattern of each individual cell in response to the mean elastic stress produced by the ensemble of cells involves an overall change in the *magnitude* of the force exerted by the cells on the medium (i.e., changes in the size and/or number of the stress fibers and focal adhesions); it does not involve a change in the *orientation* of the cells.

However, in cases where the elastic field is not symmetric, for example due to nonsymmetric tractions along the

different gel boundaries, cells self-polarize preferentially along one direction, the stiffer direction [4,19,24]. In a rectangular gel that was held at two opposite boundaries and was free at the other two, fibroblasts were observed to *self-polarize* along the stiffer direction, namely, normal to the fixed boundaries [19]. In another experiment, fibroblasts were seen to spontaneously self-polarize perpendicular to a boundary with a more rigid matrix, and parallel to a boundary with a softer matrix [24].

Furthermore, elastic interactions between cells can also affect the local organization of cells [4]. For example, it was observed that cells often align head to tail, forming stringlike structures [3,17], where each cell orients along the tensile field produced by its neighbors. In a recent study by Nelson *et al.* [11], in which patches of cells with well-defined shapes were deposited on a two-dimensional gel, a spontaneous patterning of the cells was observed. These observations are important because cellular patterning, and polarization in particular (which involves a morphological change in the cellular cytoskeleton), was shown to correlate with other cellular processes such as cell division [1,3,11,17,19]. In addition, the experiments of Nelson *et al.* [11] showed that the level of cell proliferation, and the forces the cells exert, depended on the shape of the cellular domain. The authors concluded that the long-range elastic interactions between the cells are responsible for the observed shape dependence.

In the absence of external forces, a composite system of cells and gel is isotropic (and homogeneous on scales much larger than the cell size), since we consider systems in which the cells are uniformly distributed and are either randomly oriented or appear with rounded morphologies [18,23]. The spatially averaged, mechanical response of such an isotropic cell-gel system can be characterized by two independent, measurable parameters [25]. These so-called elastic susceptibilities, denoted here by χ_s and χ_v, reflect the distinctive response of a particular cell type to pure shear and pure volume deformations, respectively. Cells that comprise tissues (and in some *in vitro* experiments as well) are often segregated and organized in well-defined domains that may be elastically distinct from their surroundings. An important example is a tumor that is composed of cancer cells and an extracellular matrix that is elastically distinct from the surrounding tissue; this often forms the basis of tumor diagnosis [1]. Other examples include fibroblasts in tendons, smooth muscle and endothelial cells in blood vesicles, or cell aggregates in artificial gels [26,27]. The mechanical response of the cells in these cases is dictated not only by their characteristic elastic susceptibilities (χ_s and χ_v), but also by the geometry and elasticity of the surrounding matrix, which determine the local elastic field. Since the cellular forces constantly adjust to stress fields that are, in part, determined by these forces, a theory that predicts the resulting stationary behavior of the system requires a self-consistent calculation.

In this paper, we present a quantitative theory that predicts the self-polarization of cells in cell-gel domains that are embedded in a cell-free matrix (see Fig. 1), as a function of the domain shape and its elastic properties relative to its surroundings. For simplicity, we focus on spheroidal domains; in that case, the elastic strain field developed inside the domains is constant (on length scales much larger than the cell size). The dependence of the field in a polarizable domain on the domain shape is given in electrostatic (magnetostatic) theory by the depolarization (demagnetization) factors [28]. In elastic systems, the Eshelby tensor plays an analogous role [29]. Our understanding of the properties of active cells in cellular domains is based on a generalization of the known results for passive inclusions in solids to include the effects of cell activity. We predict phenomena that are unique to systems with feedback and self-regulation, such as the self-polarization of cells. In particular, we show that the spontaneous polarization of the cellular forces (as determined by the direction of their focal adhesions and stress fibers) is determined by the domain shape. Our results are consistent with experiments that demonstrated the self-polarization of cells in anisotropic mechanical environments [11,19], as well as an experiment by Eastwood *et al.* [17] that measured the dependence of cell polarization on the aspect ratio of a rectangular gel. The differences in the development of cellular force in fixed and floating gels [23] (see above) may also be quantified by our model. Finally, we show that the relation between the two elastic susceptibilities χ_s and χ_v reflects different mechanisms of cell polarization. We discuss two plausible mechanisms for cell polarization: orientational polarization and axially induced polarization (see Fig. 2). We outline their distinctive consequences and predict their effects on experiments.

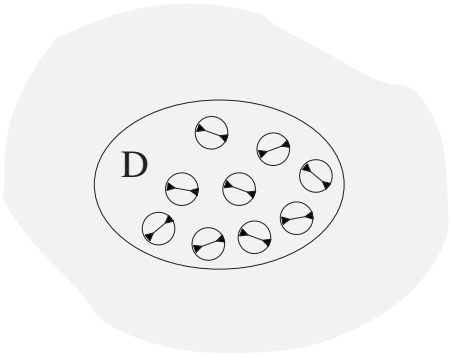

FIG. 1. Schematic illustration of a spheroidal domain of contractile cells, incorporated in a cell-free, elastic matrix. In this case, the uniform gray color of the background indicates that the elastic, gel-like matrix in the cellular domain and the surrounding, cell-free matrix are the same material and have the same elastic moduli.

II. THEORY

For simplicity we consider a spheroidal domain comprising contractile cells (such as muscle cells, fibroblasts, or endothelial cells) in an elastic, gel-like matrix [30]; this cellular domain is embedded in a three-dimensional, cell-free [31] matrix of macroscopic dimensions (see Fig. 1 and relevant examples above). We assume that the cellular domain is small compared with the dimensions of the surrounding gel, but much larger than the cell size, so that it can be treated as a uniform or homogeneous composite material consisting of active cells embedded at some finite density in a gel. In many cases of experimental and biological interest, the elastic properties of such cellular domains are different from

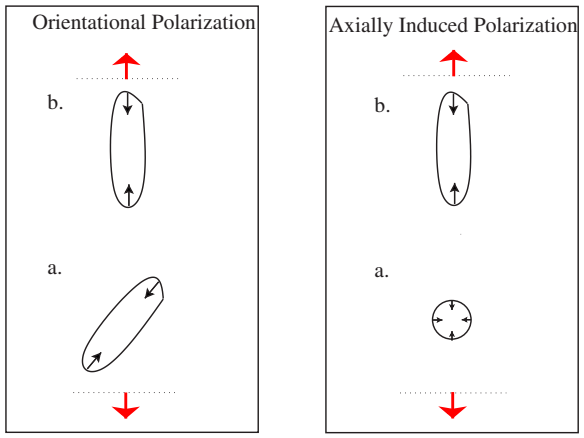

FIG. 2. (Color online) Schematic illustration of two suggested polarization mechanisms. *a* and *b* are the initial and final cell configurations. The black (thin) and red (thick) arrows show the directions of the cellular stress and the applied load, respectively. The left panel corresponds to $\chi_v=0$ and the right to $\chi_v=\chi_s$ (see Appendix A).

those of their surroundings. Nevertheless, one can, in principle, synthesize the surrounding gel to have any desired elastic properties. To simplify the discussion below, we first consider the idealized situation in which the cellular domain and its surroundings are characterized by the same elastic moduli—in general, this is denoted by a fourth-rank tensor **C**. Afterward, we generalize this theory to treat the more complex case in which the elastic moduli of the cellular domain and its surroundings are different.

As will be shown below, a domain containing contractile cells that pull on their surroundings acts in a manner that is similar, in some respects, to an elastic (misfit) inclusion in a homogeneous solid. However, there are important differences that make the case of cellular inclusions unique compared with the case of passive inclusions: the forces produced by the cells are constantly regulated and modified in response to environmental stimuli, such as the local strain or stress field. Thus, unlike passive inclusions, a domain containing contractile cells embedded in an elastic matrix can actively *self-polarize* in the elastic field that arises from the traction forces [19,23] produced by the cells themselves. This will be discussed in more detail below.

The long-range, elastic deformations caused by the contractile activity of each cell are determined by the contribution of the force dipole tensor, $p_{ij}=\Sigma f_i l_j$, where $\vec{f}$ and $\vec{l}$ are the force at, and the radius vector to, each adhesion contact of the cell with its surroundings; the sum is over all such contacts [5]. The cellular dipole tensor p_{ij} is not necessarily a constant. Many cell types modulate both the size and orientation of their focal adhesions and stress fibers in response to changes in the local stress, thereby changing the dipole tensor p_{ij}. These changes are often observed to be accompanied by global changes in the cell shape and alignment. In particular, cell polarization can be related to a change in cell shape, typically, from round to more elongated (or bipolar) morphologies [17], or by an overall reorientation of the cell major axis [18] (cf. Fig. 2). Nevertheless, while in general the cellular force pattern of contractile cells is coupled to shape changes, no systematic quantification of this relation has yet been reported. Thus, we limit our focus to theoretical predictions of changes in the average force dipole $\langle p_{ij}\rangle$ rather than to changes in cell shape. As we shall see below, measurement of the two independent cell susceptibility parameters χ_s and χ_v may allow us to distinguish between the two different polarization schemes mentioned above.

Cell polarization occurs regardless of whether the polarizing field is externally applied [17,18], or internally generated by the cells themselves [19,23]. To quantitatively account for the cellular polarization response in different experimental situations, it is important to define an appropriate reference state with respect to which the changes in the cellular dipoles should be measured. Contractile cells exert finite forces even in the absence of any external forces [23]. We designate the corresponding, force-free value of the dipole tensor of each cell by p_{ij}^0. The force-free situation is relevant for the case where the cellular domain is freely floating in solution and not surrounded by an elastic material; in this case, the system can freely adjust to the forces produced by the cells. The resulting value of the dipole tensor defines the reference state quantity p_{ij}^0 of each cell. In the rest of this paper, we focus on the effects that arise when the force-free cellular domain is embedded in a macroscopically large elastic matrix. We calculate the change in the mean cellular dipole tensor $\langle p_{ij}\rangle$, compared with the reference state in which each dipole has the value p_{ij}^0. Since we assume that in the reference state the cells are randomly distributed, the reference state domain will deform uniformly and isotropically until mechanical equilibrium is reached in which the cellular forces are balanced by the stresses due to the surrounding elastic medium. In the reference state, the average ("hydrostatic") stress σ_{ij}^0 and strain u_{ij}^0 produced by the cells are given by

$$\sigma_{ij}^0=\rho\langle p_{ij}^0\rangle=\frac{\rho p^0}{3}\delta_{ij} \quad \text{and} \quad u_{ij}^0=\mathbf{C}^{-1}\sigma_{ij}^0=\frac{\rho p^0}{9\kappa}\delta_{ij}, \quad (1)$$

where p^0 is the average dipole strength in the ensemble of cells, and κ is the bulk modulus of the gel in which the cells are embedded. Here and everywhere below, we use boldface letters to designate fourth-rank tensors and a product of the form $\mathbf{A}g_{ij}$ denotes $A_{ijkl}g_{kl}$. Similarly, $\mathbf{AB}g_{ij}=A_{ijmn}B_{mnkl}g_{kl}$, where summation over repeated indices is implied. The magnitude of the isotropic stress in the reference state is given by the product of the number of cells per unit volume, ρ, and the magnitude of the average compressional stress per cell, which is proportional to p^0.

Since the elastic deformations of the cells are long range, the elastic interactions between the cells are mediated by both the gel of the cellular domain and the surrounding, cell-free, elastic matrix. The presence of the surrounding matrix restricts the allowable deformation of the cellular domain and provides a restoring force that, like an applied field, can give rise to an active *self-polarization* of the cells. Furthermore, since the propagation of the elastic stress in the surroundings and within the cellular domain depends on the shape of the cellular domain, the extent of self-polarization

of the cells is also shape dependent. This effect is a direct consequence of the long-range nature of the elastic interaction between the cells.

We define u_{ij}^c as the average strain produced by the traction forces arising from the entire ensemble of cells, and u_{ij}^a as an additional strain that results from externally applied forces. Together, these sources give rise to an *excess* strain (relative to the reference state) that we denote as u_{ij}, where

$$u_{ij} = u_{ij}^{tot} - u_{ij}^0 = u_{ij}^a + u_{ij}^c - u_{ij}^0 \tag{2}$$

(recall that $u_{ij}^0 = \rho \mathbf{C}^{-1} \langle p_{ij}^0 \rangle$). The excess strain may give rise to active changes in the cellular force pattern that modify the magnitude and orientation of the cellular dipolar tensor; these result in self-polarization of the cells. For weak enough fields, we can assume that, on the average, the polarization response of the cells is linearly related to the excess strain [32]; this is stated mathematically as follows [25]:

$$P_{ij} = \rho[\langle p_{ij} \rangle - \langle p_{ij}^0 \rangle] = -\boldsymbol{\chi} \sigma_{ij} = -\boldsymbol{\chi} \mathbf{C} u_{ij}, \tag{3}$$

where P_{ij} is termed the polarization stress (in analogy to the polarization field in electrostatics), and $\sigma_{ij} = \sigma_{ij}^{tot} - \sigma_{ij}^0 = \mathbf{C} u_{ij}$ is the excess stress, relative to the reference state. The fourth-rank tensor $\boldsymbol{\chi}$ is the cellular susceptibility tensor, which accounts for the active response of the cell (and the reorganization of its focal adhesions and stress fibers) in the presence of a local stress. This tensor contains all the effects of the elastic interactions among the cells that influence the polarization response of the cells to a local elastic field—whether it be applied or internally generated—above and beyond those that exist in the reference state. In a recent paper [25], we presented a microscopic, statistical mechanics theory from which the susceptibility tensor could be related to other known properties of the cell and the matrix. In general, $\boldsymbol{\chi}$ depends on the particular type of cell and the elastic properties of the cellular domain; the symmetry characteristics of $\boldsymbol{\chi}$ reflect the mechanism by which the cells polarize (see Fig. 2 and Appendix A).

Equation (3) shows that the average of the cellular dipole tensor $\langle p_{ij} \rangle$ depends on the excess strain in the medium. The latter [see Eq. (2)], in turn, depends on the cellular strain and hence on $\langle p_{ij} \rangle$ itself. To evaluate $\langle p_{ij} \rangle$ self-consistently, we first calculate the strain field (or the deformation) u_{ij}^c due to the deformations induced by the force dipoles that are localized within the cellular domain; however, this expression gives the strain in the entire sample. This is done by integrating the strain contributions from all the dipoles within the cellular domain D, as follows:

$$u_{ij}^c(\mathbf{r}) = \rho \int_D G_{il,jk'}(\mathbf{r}, \mathbf{r}') \langle p_{lk}(\mathbf{r}') \rangle d^3\mathbf{r}'$$

$$= \rho \langle p_{lk} \rangle \int_D G_{il,jk'}(\mathbf{r}, \mathbf{r}') d^3\mathbf{r}', \tag{4}$$

where the tensor $G_{ij}(\mathbf{r}, \mathbf{r}') = G_{ij}(|\mathbf{r} - \mathbf{r}'|)$ is the Green's function [33] for a point force in an infinite, three-dimensional, homogeneous, and isotropic medium [34]. The set of subscripts that follow the comma in Eq. (4), j and k', denote differentiation of the Green's function with respect to $\vec{r}$ and $\vec{r}\,'$, respectively. The second equality in Eq. (4) arises from the assumption that the cells are distributed homogeneously, so that, within the cellular domain, the average of the dipole tensor is position independent. The subscript D on the integrals denotes that the integration is only over the volume of the domain that contains the cells and not over the surrounding, cell-free matrix.

The integral in Eq. (4) has been calculated for ellipsoidal domains by Eshelby [35]. The strain *inside* the cellular domain may be expressed in terms of the Eshelby tensor $\mathbf{S}$ as follows:

$$u_{ij}^c = \rho \mathbf{S} \mathbf{C}^{-1} \langle p_{ij} \rangle \tag{5}$$

[36]. Sufficiently far from the domain boundary, $|\mathbf{r}|/|\mathbf{r}_D| \to \infty$, the strain field due to the cells, $u_{ij}^c(\mathbf{r})$, vanishes [37] and only the applied field u_{ij}^a remains. Inside the domain, namely, $\mathbf{r} \in D$, $u_{ij}^c(\mathbf{r})$ is a constant [35]. This result is consistent with the assumption expressed in the second equality of Eq. (4) that the domain is uniformly polarized.

The susceptibility tensor [see Eq. (3)] allows us to express the cellular strain u_{ij}^c in terms of the reference state strain $u_{ij}^0 = \rho \mathbf{C} \langle p_{ij}^0 \rangle$ and the excess strain $u_{ij} = u_{ij}^{tot} - u_{ij}^0$; we find $u_{ij}^c = \mathbf{S}(u_{ij}^0 - \boldsymbol{\chi} u_{ij})$. Substituting that relation into Eq. (2) results in the following self-consistent equation for the total strain field u_{ij}^{tot} in the cellular domain:

$$u_{ij}^{tot} = u_{ij}^a + \mathbf{S}[u_{ij}^0 - \boldsymbol{\chi}(u_{ij}^{tot} - u_{ij}^0)]. \tag{6}$$

Solving for u_{ij}^{tot}, we obtain

$$u_{ij}^{tot} = \mathbf{A}[u_{ij}^a + \mathbf{S}(\mathbf{I} + \boldsymbol{\chi}) u_{ij}^0], \tag{7}$$

where

$$\mathbf{A} = (\mathbf{I} + \mathbf{S}\boldsymbol{\chi})^{-1} \tag{8}$$

and $\mathbf{I}$ is the fourth-rank symmetric unit tensor [38].

The overall strain in the cellular domain is now expressed in terms of the applied strain and the cellular strain in the reference state [cf. Eq. (1)]. Both these quantities can be measured in separate experiments. The contribution of cell interactions is incorporated in the susceptibility tensor $\boldsymbol{\chi}$, which can depend on the concentration of cells [25], and its dependence on the shape of the cellular domain enters through the Eshelby tensor $\mathbf{S}$. Note that, even if u_{ij}^0 were zero, the cells could still polarize (in principle) due to the applied strain, and would then contribute to the total strain through the dependence of $\mathbf{A}$ on $\boldsymbol{\chi}$. However, without the intrinsic cell contractility (namely, if $p_{ij}^0 = u_{ij}^0 = 0$) the cells cannot self-polarize [see Eqs. (9) and (10) below].

As noted above, an active cellular domain is distinguished from a passive inclusion in a solid by its ability to actively polarize. By setting the susceptibility tensor to zero, $\boldsymbol{\chi} = 0$, we recover the familiar result of Eshelby [35], $u_{ij}^c = u_{ij}^{tot} - u_{ij}^a = \mathbf{S} u_{ij}^0$ relating the free transformation strain u_{ij}^0 of the inclusion in solution to the actual strain it produces in the elastic matrix, u_{ij}^c. Thus, the ability of the "inclusion" to polarize requires our generalization of the usual theory of passive inclusions to include the active nature of cellular elastic dipoles.

Furthermore, by substituting Eq. (7) in Eq. (2) and using the relation $\mathbf{A}^{-1}=(\mathbf{I}+\mathbf{S}\boldsymbol{\chi})$ above we find $u_{ij}=u_{ij}^{tot}-u_{ij}^{0}=\mathbf{A}[u_{ij}^{a}+(\mathbf{S}-\mathbf{I})u_{ij}^{0}]$; inserting that into Eq. (3) we obtain an expression for the cellular polarization stress:

$$P_{ij}=-\boldsymbol{\chi}\mathbf{C}\mathbf{A}[u_{ij}^{a}+(\mathbf{S}-\mathbf{I})u_{ij}^{0}]. \tag{9}$$

This equation predicts an interesting effect: the cells polarize due to the traction forces that they themselves exert on the surrounding matrix; this occurs even in the absence of external stress. That is, there is a nonzero value of P_{ij} even when $u_{ij}^{a}=0$. This response, which we call self-polarization, is given by the second term in Eq. (9):

$$P_{ij}^{self}=-\frac{\rho p^{0}}{9\kappa}[\boldsymbol{\chi}\mathbf{C}\mathbf{A}(\mathbf{S}-\mathbf{I})]\delta_{ij}. \tag{10}$$

Generalization of the model: Cellular domain and its surroundings have different elastic moduli

The results presented above are readily generalized for situations in which the average elastic moduli of the cellular domain are different from those of the surrounding matrix. This problem is similar, but not identical, to the so-called inhomogeneous inclusion problem in solids [39], in which an elastic inhomogeneity does not perfectly fit (in size or shape) within the solid, and exerts a force on the inclusion-matrix boundary. The difference between the cellular case and the case of a "dead" inclusion arises, as noted previously, from the ability of the cells to actively respond to stresses that can arise, at least in part, from forces generated by the cells themselves. The generalization described in this section is important for many realistic situations in which a tissue with one cell type is surrounded by another tissue with different elastic moduli. The limiting cases in which the elastic moduli of the surrounding matrix are either zero or infinity describe the situation of free (e.g., floating) or fixed (e.g., glued to a rigid surface) cellular domains, respectively (cf. Ref. [23]) (for cellular domains with no surrounding matrix).

We denote the elastic constants of the cellular domain and of the surrounding matrix by $\mathbf{C}_c$ and $\mathbf{C}_m$, respectively. The calculation of u_{ij}^{c} by use of the Green's function for an isotropic and homogeneous medium, as in Eq. (4), no longer holds because the system is no longer homogeneous. This difficulty is overcome by using the so-called equivalent inclusion method of Eshelby [35,39]. To this end, we first perform the previous calculations for an artificial, homogeneous system that has the elastic moduli $\mathbf{C}_m$ appropriate to the matrix. The excess field in the cellular domain D due to the difference in the elastic moduli of the cellular domain and the surroundings is then accounted for by introducing an *artificially polarizable* inclusion in D, which has the matrix elastic moduli $\mathbf{C}_m$, and exerts a force on the boundary of the cellular domain D [39].

Since we consider ellipsoidally shaped domains, the excess field in the domain D is uniform [35]; and if, for instance, D is stiffer than its surroundings, the strain within it is smaller by a constant amount, and vice versa. This excess strain is "induced" only when the cellular domain is subject to a force from the surroundings (that originates from either the active forces of the cells or from external sources). The magnitude of this strain is proportional to the difference between the elastic moduli of the cellular domain and the surroundings, $\mathbf{C}_c-\mathbf{C}_m$. For a given average value of the cellular dipole $\langle p_{ij}\rangle$, the total restoring stress exerted by the surroundings on the cellular domain is $\sigma_{ij}^{tot}-\rho\langle p_{ij}\rangle=\sigma_{ij}-P_{ij}$, where $\sigma_{ij}^{tot}=\mathbf{C}^{-1}u_{ij}^{tot}$ is the total stress (since $\rho\langle p_{ij}\rangle$ would be the domain stress in the absence of the matrix).

Denoting the excess strain due to the difference between the elastic moduli of the cellular domain and the surrounding matrix (the excess inhomogeneity strain) by u_{ij}^{ih}, and the induced polarization stress that (artificially) generates this strain by P_{ij}^{ih}, we have [39] [cf. Eqs. (3) and (5)]

$$u_{ij}^{ih}=\mathbf{S}_m\mathbf{C}_m^{-1}P_{ij}^{ih} \tag{11}$$

and

$$P_{ij}^{ih}=-\boldsymbol{\chi}^{ih}(\sigma_{ij}-P_{ij}), \tag{12}$$

where

$$\boldsymbol{\chi}^{ih}=(\mathbf{C}_c-\mathbf{C}_m)\mathbf{C}_m^{-1} \tag{13}$$

and the subscript on $\mathbf{S}_m$ indicates that the Eshelby tensor is evaluated using the elastic moduli of the surrounding, cell-free matrix. The tensor P_{ij}^{ih} is known in the theory of composites as the polarization stress [39]. This tensor is a mathematical device that reflects a purely mechanical and generally fast response of the medium. This is in contrast with the polarization response P_{ij}, which reflects an active biological response of the cells to applied forces; these may take hours and even days to be established, since they involve the reorganization of the focal adhesions and stress fibers. Thus, experiments done on a short time scale would show only the passive elastic response of the cellular domain, while those done on long time scales would also measure the effects due to the active polarization response.

Including the inhomogeneity field u_{ij}^{ih} in the total strain, $u_{ij}^{tot}=u_{ij}^{a}+u_{ij}^{c}+u_{ij}^{ih}$, one may repeat the previous derivation to find generalized expressions for the total strain u_{ij}^{tot} and cell polarization P_{ij} for the case where the cellular domain and surrounding matrix have different elastic properties. We find

$$u_{ij}^{tot}=\mathbf{A}[u_{ij}^{a}+\mathbf{S}_m(\mathbf{I}+\boldsymbol{\chi})(\mathbf{I}+\boldsymbol{\chi}^{ih})u_{ij}^{0}] \tag{14}$$

and

$$P_{ij}=-\boldsymbol{\chi}\mathbf{C}_c\mathbf{A}[u_{ij}^{a}+(\mathbf{S}_m-\mathbf{I})u_{ij}^{0}]. \tag{15}$$

The strain in the reference state $u_{ij}^{0}=(\rho p^{0}/9\kappa_c)\delta_{ij}$ is calculated using the bulk modulus of the cellular domain. The calculations yield a generalization of the expression for $\mathbf{A}$:

$$\mathbf{A}=\{\mathbf{I}+\mathbf{S}_m[(\mathbf{I}+\boldsymbol{\chi})\mathbf{C}_c-\mathbf{C}_m]\mathbf{C}_m^{-1}\}^{-1}. \tag{16}$$

In the usual problem of elastic inhomogeneity, the tensor $\mathbf{A}=[\mathbf{I}+\mathbf{S}_m(\mathbf{C}_c-\mathbf{C}_m)\mathbf{C}_m^{-1}]^{-1}$ (which is often termed the stress-concentration tensor) relates the strain inside an inhomogeneity to the applied strain through $u_{ij}^{tot}=\mathbf{A}u_{ij}^{a}$. This is the situation for passive inhomogeneities, applicable to noncontractile cells that do not actively exert forces, and hence both the reference state strain u_{ij}^{0} and the active susceptibility $\boldsymbol{\chi}$

are zero. If only $\boldsymbol{\chi}$ is zero but not u_{ij}^0 (corresponding for instance to the case that the cells are active but the measurement is performed on a time scale that is too short to allow them to respond), the system describes a special case of a passive inhomogeneous inclusion (namely, an inclusion that misfits in shape or size in the matrix and whose elastic properties are different from those of the surroundings), and u_{ij}^0 is the free transformation strain of the inclusion [39]. However, for the case of active inclusions relevant to biological cells, the equations describing passive inclusions no longer apply. Equation (16) shows that the overall elastic response of a system with active, contractile cells can be described by an effective set of elastic moduli [25], given by $\tilde{\mathbf{C}}_c=(\mathbf{I}+\boldsymbol{\chi})\mathbf{C}_c$; in that case, the tensor $\mathbf{A}$ can be interpreted as an *effective* stress-concentration tensor. Note that, even for the simple case in which the elastic moduli of the cells and the matrix are identical (i.e., $\mathbf{C}_c=\mathbf{C}_m$), the cellular domain behaves like an elastic inhomogeneity on long time scales; and one may replace $\boldsymbol{\chi}$ by $(\tilde{\mathbf{C}}_c-\mathbf{C}_c)\mathbf{C}_c^{-1}$ in Eqs. (8) and (16).

III. RESULTS AND DISCUSSION

An important prediction of our theory is that an ensemble of contractile cells localized in an asymmetrically shaped domain, which is surrounded by a compliant matrix, will spontaneously polarize in a particular direction, due to the anisotropic stresses exerted on the cellular domain by the surrounding matrix. The phenomenon of self-polarization is often encountered in experiments with active cells, in both two and three dimensions; for example, in [11,17,19]. Nelson *et al.* [11] observed a spontaneous patterning of cells within microfabricated patches of cells that were deposited on a two-dimensional gel. The authors also found that the forces produced by the cells depended on the shape of the microfabricated patch of cells. Takakuda and Miyairi [19] examined rectangular, cell-populated gels and used a combination of fixed and free boundaries to produce an anisotropic field in the samples; however, no external forces were applied. In these experiments the cells spontaneously polarized in parallel to the stiffer direction (perpendicular to the fixed boundaries). Our theory considers the effects of an ensemble of polarizable, force dipoles to model the mechanical activity of cells and provides a physical and quantitative explanation for the phenomena of self-polarization. We focus on a particular case in which the shape of the cellular domain is the cause of the anisotropy of the elastic field. In other systems, for instance, the experiment of Takakuda and Miyairi, the anisotropy of the field is produced by the boundary conditions. In real tissues the elastic fields are anisotropic as a rule and not as an exception; thus this physical mechanism of self-polarization may play an important role for the self-organization of tissues.

The prediction of self-polarization for an ensemble of dipoles is unique to the elastic system in which the force dipole is a tensor of rank 2 and not a vector. An analogous effect does not occur for the vector dipoles of electrostatics or magnetostatics, where the field due to opposing dipoles sums to zero, and thus an isotropic ensemble of dipoles (e.g., a dielectric) produces a vanishing macroscopic field in the absence of an external field. In contrast, an isotropic ensemble of force dipoles does produce a finite strain in the absence of an external field; it is the isotropic reference-state strain $u_{ij}^0=(\rho p^0/9\kappa_c)\delta_{ij}$. No symmetry breaking occurs, however, in the absence of a surrounding matrix. The spontaneous symmetry breaking, which leads to cell self-polarization, arises from the anisotropic elastic restoring force exerted by the surrounding matrix on the anisotropically shaped cellular domain. This effect, however, does not occur for a completely incompressible ($\kappa_c\to\infty$) cellular domain. While some materials are indeed close to the incompressible limit, on the time scale of hours or even days relevant to cellular systems, cellular domains can often be regarded as compressible since experiments show that cells actively compress the gels in which they are placed [40,41]. In the limiting cases that the surroundings are infinitely rigid (fixed boundaries), or infinitely soft (free boundaries), no symmetry breaking (i.e., anisotropic polarization) is expected; since in the latter case the restoring force due to the surroundings is perfectly isotropic and in the former it is zero. Nevertheless, in the case of fixed boundaries, the dipolar strength of each cell may increase to a maximum value, depending on the mechanism of cell polarization, as explained below.

A. Shape dependence

To illustrate the shape dependence of the spontaneous polarization induced by the surrounding matrix we consider spheroidal cellular domains whose principal axes are oriented parallel to a Cartesian coordinate system [42]; i.e., the surface of the domain is given by the equation

$$\frac{x^2}{a^2}+\frac{y^2}{a^2}+\frac{z^2}{c^2}=1. \tag{17}$$

The shape of the domain is then uniquely determined by the aspect ratio $r=c/a$.

Figure 3 shows the (normalized) xx (dashed, red) and zz (dot-dashed, green) elements of the self-polarization tensor P_{ij}^{self} and its trace (solid, black), as a function of the aspect ratio. The left and right panels are for two distinct cellular polarization mechanisms that are schematically illustrated in Fig. 2 and defined in Appendix A. The right panel is for a pure orientational polarization mechanism, in which only the orientation of the cellular dipole changes but its magnitude is fixed. The left panel is for an axially induced mechanism, in which the cellular stress enhances in the direction of the excess stress (relative to the reference state). These two mechanisms correspond to different values of the two components of the susceptibility tensor χ_v and χ_s. Recall that the self-polarization tensor defined, for the case of zero applied stress ($\sigma_{ij}^a=0$), by $P_{ij}^{self}=\rho[\langle p_{ij}\rangle-\langle p_{ij}^0\rangle]$, is a measure of the increase of the cellular dipole relative to the reference state in which the cells are isotropically distributed in a free sample. In Appendix B we provide analytic equations for the elements of the self-polarization tensor and the total strain u_{ij}^{tot} for three special values of $r=c/a$: disks ($r\to 0$), spheres ($r=1$), and rods ($r\to\infty$).

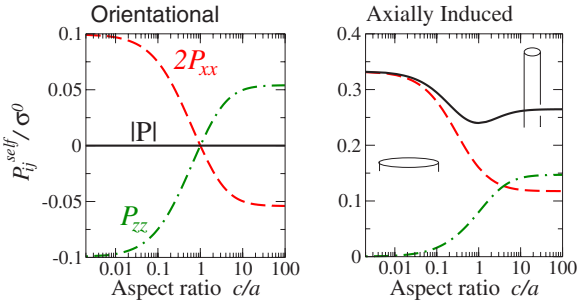

FIG. 3. (Color online) Self-polarization of cells as a function of the aspect ratio of the cellular domain, compared for the two polarization mechanisms (see Fig. 2 and Appendix A): Orientational polarization (χ_s=0.5, χ_v=0), left panel, and axially induced polarization (χ_s=0.5, χ_v=0.5), right panel. The red (dashed), green (dot-dashed), and black (solid) curves are for the xx and zz elements of the self-polarization tensor, and its trace, P_{ii}^{self}, respectively. The y axis is normalized by the value in the reference state, $\sigma^0=\sigma_{xx}^0=\sigma_{zz}^0=\rho p^0/3$. In all these cases, the elastic constants of the cellular domain and the surrounding, cell-free, matrix are identical; the Poisson ratios are $\nu_c=\nu_m=0.4$.

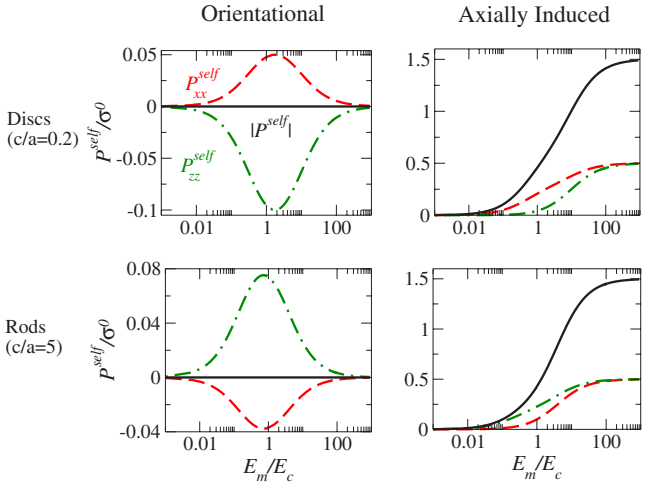

FIG. 4. (Color online) Self-polarization of cells as a function of the ratio of the Young's modulus of the surrounding matrix and that of the cellular domain. We plot the value of the self-polarization for the two polarization mechanisms discussed in Appendix A; and for two generic shapes of the cellular domain. Upper panels are for disklike domains (c/a=0.2), and lower panels are for rodlike domains (c/a=5). The red (dashed), green (dot-dashed), and black curves are the xx, and zz components and the trace of the self-polarization tensor P_{ii}^{self}, respectively. The Poisson ratios of the matrix and the cellular domain are ν_m=0.5 and ν_c=0.3, respectively.

A prediction that is common to both panels of Fig. 3 is that the cells tend to polarize parallel to the long axis of the spheroid. We first consider the orientational polarization case (left panel). The black curve shows that there is no change in the magnitude of the mean cellular dipole, as assumed by considering χ_v=0. For oblate (disk-shaped) spheroids, P_{zz}^{self} is negative, meaning that the mean cellular traction along the z axis is smaller than in the reference state; the traction along the x axis is higher and therefore $P_{xx}^{self}>0$. The opposite behavior is predicted for prolate (rodlike) spheroids. By symmetry, in a spherical domain there is no net polarization.

In contrast, for the axially induced case where χ_v is nonzero (and we take as an example $\chi_s=\chi_v=1/2$), the dipole strength of each cell increases monotonically compared with its value in the reference state; this is true even for spherical domains, due to the force exerted by the surrounding (cell-free) matrix. In the case of axially induced polarization, the value of the mean cellular dipole is lowest in the random reference state, and therefore all curves on the right panel of Fig. 3 for the polarization relative to the reference state are positive.

In an experiment by Eastwood *et al.* [17], two configurations of a rectangular fibroblast-populated gel were examined. By restraining the gel at either its opposing long or short edge they effectively (and respectively) produced low- and high-aspect-ratio domains. Constraining the system at one end produces an asymmetry of the elastic field that is qualitatively similar to the symmetry breaking resulting in our system due to the asymmetric shape of the cellular domain. More explicitly, for a prolate domain oriented parallel to the z axis, we find $|u_{zz}^{tot}|<|u_{xx}^{tot}|$; for example, for the limiting case of a rodlike domain where the aspect ratio $r\to\infty$, we find $u_{zz}^{tot}=0$ and $u_{xx}^{tot}\sim\rho p^0<0$ [see Eq. (B8) in Appendix B]. This means that the matrix resistance acting against an axial compression across the long z axis of the spheroid is stronger than that preventing a compression of its cross section (in the x,y plane); and this is the reason that, in rodlike domains, the cells align parallel to the long axis of the domain since this is the direction in which the matrix resistance is largest. Indeed, a similar effect was obtained in the Eastwood *et al.* experiment by fixing only the short edges of the rectangular (the high-aspect-ratio configuration). Since the long axis is effectively stiffer than the perpendicular (free) direction, it resulted in a pronounced cell polarization in parallel to the long axis of the sample. As noted by Eastwood *et al.*, this prediction is also consistent with the alignment of fibroblasts in parallel to the long axis of tendon filaments.

B. Effect of matrix stiffness

The effect of the matrix stiffness differs in an essential way for the two polarization mechanisms discussed in Appendix A. For the axially induced mechanism, the cell polarization increases monotonically with the stiffness of the surrounding matrix. This is in contrast to the orientational mechanism in which the self-polarization first increases to a maximal value and then decreases to zero. This behavior is qualitatively independent of the domain shape. Figure 4 plots the variation of the spontaneous cell polarization as a function of the ratio of the Young's moduli, E_m/E_c (for two chosen values of the Poisson ratios ν_m=0.5, ν_c=0.3). The respective increases of the shear and bulk moduli are given by the usual elasticity relations $\mu=E/2(1+\nu)$ and $\kappa=E/3(1-2\nu)$.

As seen in Fig. 4, the surrounding matrix must exhibit some elastic resistance for spontaneous polarization of the cells to occur. In the absence of a restraining field from the surrounding matrix, no excess field u_{ij} develops in response to the cellular compression of the reference state u_{ij}^0, and the

cells do not self-polarize. The explicit expression of the polarization tensor is given in Eq. (15). The tensor $\mathbf{A}$ relates the field in the cellular domain to the long-range field in the surrounding matrix. If $\mathbf{A}$ is small, stress does not propagate in the surrounding matrix. In the limit that $\kappa_m/\kappa_c \to 0$ and $\mu_m/\mu_c \to 0$, which corresponds to a sample with free boundaries, it is readily verified that $\mathbf{A} \to 0$; and therefore $P_{ij} \to 0$.

This result is consistent with the behavior of fibroblasts in freely floating collagen gels [23,43]. While the cells initially compress the gel (thus, $u_{ij}^0 \neq 0$), they appear in stellate shapes and no development of stress fibers is seen. In contrast, when the same gel is glued to a surface and its surface is held fixed, well-defined stress fibers develop within the cells [23,43]. In a symmetrically round disk, the field that "induces" the development of stress fibers is isotropic in the plane and therefore only the magnitude of the cellular dipole increases, while its symmetry remains isotropic. In contrast, in a cellular domain with a large aspect ratio, as in the experiment by Eastwood *et al.* [17], the cells polarize in parallel to the long axis of the sample. In those experiments, fibroblasts changed from a more or less round configuration to a highly polarized structure, while simultaneously increasing the force in that direction. The evidence from these experiments suggests that the axially induced case is appropriate to describe the observed polarization response of those fibroblasts (Fig. 2, right panel).

The two polarization mechanisms show different trends when the rigidity of the surrounding matrix is increased. In general, more rigid matrices exert stronger restoring forces and those stronger forces give rise to the enhanced cellular adhesiveness and contractility. In the axially induced mechanism (Fig. 4, right panel), the magnitude of cell polarization increases monotonically with the relative stiffness of the surroundings up to a maximal value. In the limit that the surrounding matrix is infinitely rigid (fixed boundaries), the boundaries of the cellular domain are held by the strongest possible restoring field and thus the cell polarization reaches maximal values.

In the fixed boundary limit, the response to the initial isotropic contraction, $\langle p_{ij} \rangle = p_{ij}^0$, is also isotropic. In this case, the cellular response can therefore only be isotropic, and $P_{xx}^{self} = P_{yy}^{self} = P_{zz}^{self}$ as seen in Fig. 4. Since the boundaries are fixed (and $u_{ij}^a = 0$) the mean strain u_{ij}^{tot} is zero. This is easily verified from Eq. (14) where, in the limit $\mathbf{C}_m \gg \mathbf{C}_c$, one finds $\boldsymbol{\chi}^{ih} = -\mathbf{I}$ [see also Eq. (13)], which causes the second term on the right-hand side of Eq. (14) to vanish. In this case, the excess strain is equal and opposite to the strain in the reference state (since the forces imposed by the rigid surroundings require the total strain to be zero), $u_{ij} = u_{ij}^{tot} - u_{ij}^0 = -u_{ij}^0$, and from Eq. (3) we find $P_{ij} = (\rho p^0/3)\chi_v \delta_{ij}$. The saturation value of the self-polarization is independent of the domain shape, and depends only the volume susceptibility parameter χ_v (the response to a pure volume change), and not on the shear susceptibility χ_s.

For this same reason, there is no self-polarization for infinitely stiff surrounding matrices for the case of a pure orientational response of the dipoles where χ_v is zero and all effects are due to χ_s alone. Thus, in the case of a purely orientational response, both the magnitude of the restraining field due to the surrounding matrix and its symmetry are important. When the cellular domain is surrounded by a very stiff environment ($E_m \to \infty$), the initial isotropic pulling of the cells is counterbalanced by an isotropic field from the matrix. This field, however, cannot give rise to a pure orientational polarization, and the self-polarization vanishes in this case.

For intermediate stiffness of the surroundings we see once more, in both polarization mechanisms, the tendency of the cells to polarize along the long axis of the spheroid. For the orientational mechanism we find an optimal ratio of E_m/E_c for which the self-polarization of the cells is largest. In general, this value depends on the shape of the spheroid. The higher the aspect ratio r, or the more rodlike the domain, the greater the symmetry breaking; thus the maximum cell polarization occurs at lower values of E_m/E_c compared with more symmetric domains.

IV. CONCLUDING REMARKS

We have presented a quantitative model that provides a physical explanation for the self-polarization of cells in elastic substrates. Our theory takes into account both the mechanical forces due to the matrix as well as the active effects that arise from the reorganization of the focal adhesions and stress fibers in response to stress. A similar, but qualitative, explanation was given by Nelson *et al.* [11] for the cellular proliferation patterns observed in microfabricated patches of cells on a two-dimensional gel. In our model, self-polarization describes in a coarse-grained manner the rearrangement and buildup of new focal adhesions and stress fibers that give rise to a modification of the overall cellular dipole tensor p_{ij}; this can also include a symmetric increase of cell contractility in all directions as observed by Grinnell [23] for fibroblasts in a symmetrically fixed round sample. The changes in the cellular dipole tensor are governed by the two measurable susceptibility quantities χ_s and χ_v, which are characteristic of the cell type and the extracellular matrix [25]. Since these parameters reflect the long-time, steady-state, elastic response of a macroscopically large cell-gel system, they can be directly determined from a measurement of the corresponding effective shear modulus $\tilde{\mu} = (1+\chi_s)\mu$ and bulk modulus $\tilde{\kappa} = (1+\chi_v)\kappa$, or via any other combination of these constants, such as the effective Young's modulus and the effective Poisson ratio [25]; the material constants μ and κ reflect the short-time passive response of the system, where cell activity has not yet had enough time to be established. To observe a spontaneous alignment of the cells, the system must exhibit some mechanical asymmetry. In our model, this occurs because the cells are embedded in (nonspherical) spheroidal domains. Measurement of the susceptibility parameters, on the one hand, and of the mean cellular force or the strain within the cellular domain, on the other hand, can provide a direct test of the predictions shown in Figs. 3 and 4, which correlate the dependence of cell self-polarization on the domain shape and the cell and matrix elastic properties. Finally, we note that the choice of spheroidal geometry was made for mathematical convenience, but a similar approach could be taken to examine more sophisticated and realistic cases in which the local strain field changes with position, as appropriate for cells in blood vesicles.

ACKNOWLEDGMENTS

We thank I. B. Bischofs, R. De, D. H. Wagner, V. A. Mironov, and N. Gov for many fruitful discussions. S.A.S. acknowledges the support of the Schmidt Minerva Center, an E.U. Network grant (SOFTCOMP), and a grant from the Israel Science Foundation. This research was made possible in part by the generosity of the Harold L. Perlman foundation.

APPENDIX A: THE SUSCEPTIBILITY TENSOR AND DIFFERENT POLARIZATION MECHANISMS

The susceptibility tensor that relates the average force dipole density to the local stress via $P_{ij}=-\boldsymbol{\chi}\sigma_{ij}$ [Eq. (3)] expresses the active response of the cells to the *excess* stress in the matrix, relative to the disordered, isotropic reference state. Since the reference state is isotropic, $\boldsymbol{\chi}$ must be an isotropic tensor and (like the elastic moduli of isotropic materials) it is determined by only two scalar quantities:

$$\chi_{ijkl}=\frac{1}{3}(\chi_v-\chi_s)\delta_{ij}\delta_{kl}+\chi_s I_{ijkl}, \tag{A1}$$

where I_{ijkl} [38] is the fourth-rank unit tensor. The parameter χ_v describes the response of the cellular force dipoles to a pure volume change or isotropic pressure, $P_{ii}=-\frac{1}{3}\chi_v\sigma_{ii}$, while χ_s is the response function for a pure shear deformation, $P_{ij}=-\chi_s\sigma_{ij}$ for $i\neq j$. A similar expression holds for $\boldsymbol{\chi}^{ih}$ [Eq. (13)], with the two parameters $\chi_v^{ih}=(\kappa_c-\kappa_m)/\kappa_m$ and $\chi_s^{ih}=(\mu_c-\mu_m)/\mu_m$. The susceptibility tensor can also be expressed in terms of an equivalent set of parameters, namely, $\chi_{\parallel}=\chi_{iiii}$ and $\chi_{\perp}=\chi_{iijj}$. These elements of the susceptibility tensor reflect, respectively, the parallel and perpendicular cellular force response to the elastic field. At the more microscopic level, they determine the ensemble average (over the entire cell population) of the extent of newly assembled stress fibers and focal adhesions parallel and perpendicular to the corresponding strain direction, respectively. Using Eq. (A1) one finds $3\chi_{\parallel}=\chi_v+2\chi_s$ and $3\chi_{\perp}=\chi_v-\chi_s$.

Interestingly, the relation between χ_s and χ_v may allow one to distinguish experimentally between different mechanisms of cell polarization. Special choices of these parameters correspond to different physical interpretations of the polarization mechanism. In this appendix, we analyze two such mechanisms as explained below and schematically illustrated in Fig. 2.

(a) Orientational polarization. In this case, only the orientational distribution of the cells changes in response to an excess stress (relative to the reference state), but the magnitude of the force dipole of each cell remains unchanged. If we idealize the cell by an anisotropic force dipole $p_{ij}=pn_in_j$, only the dipole orientation $\hat{n}$ changes, but not the dipole strength p. This may be applicable to bipolar cells, such as muscle cells, that produce a given amount of force at any given orientation; the magnitude of the force does not change with applied stress, but its direction can change. Thus these cells may polarize by changing their orientation [18]. Since the system is initially isotropic, subjecting it to an orientationally symmetric external stress (such as that produced by hydrostatic pressure) does not induce cell alignment in any particular direction and has no effect on the polarization. We thus conclude that for this case [25]

$$\chi_v=0. \tag{A2}$$

In the rotational polarization scheme $\chi_{\parallel}=-2\chi_{\perp}$, which indicates that a parallel enhancement of the force is compensated by equivalent reduction in the force in the transverse direction.

(b) Axially induced polarization. This type of polarization is analogous to the electronic polarization of (nonpolar) dielectrics. In this case, the magnitude of the induced force due to the focal adhesions and stress fibers can change in response to an excess (applied) stress. Higher applied stress results in more and larger adhesions and stress fibers, but only in the direction of the excess (applied) field (hence $\chi_{\perp}=0$). This effect can be summarized by writing the polarization response as $P_{ij}=\chi\sigma_{ij}$ [where χ is a scalar and not a fourth-rank tensor as in Eq. (3)]. In this case,

$$\chi_v=\chi_s=\chi. \tag{A3}$$

Microscopically, this describes a situation in which all cells polarize in the same direction, while the magnitude of their force dipole increases in size. In this case, there will be a response to an isotropic (volumetric) deformation which will result in the development of focal contacts and stress fibers in random directions within the cell, and therefore in a uniform increase in the dipolar stress in all directions, as seen, for example, in fibroblasts in a fixed gel [23] (see below).

The distinction between the orientational and axially induced polarization mechanisms is useful for picturing the response of cells to elastic forces in two limiting cases. However, experimental measurements in real systems could, in principle, result in any set of values for χ_v and χ_s. In the body of the paper, we present predictions for these two idealized mechanisms and relate them to available experiments.

APPENDIX B: ANALYTIC EQUATIONS FOR DISKS, SPHERES, AND RODS

For the three limiting cases of a spheroid, the disk, sphere, and rod, the Eshelby tensor assumes a simple form [39]. This allows us to write simple expressions for important quantities of interest, such as the self-polarization tensor P_{ij}^{self} and the total strain u_{ij}^{tot} (for zero applied field, $u_{ij}^{a}=0$). For the limiting cases of an infinite rod, $r\to\infty$, and an infinite disk, $r\to 0$, we find $u_{zz}^{tot}=0$ and $u_{xx}^{tot}=u_{yy}^{tot}=0$, respectively; these results are independent of the elastic nature of the matrix (or the cellular domain) because, in these cases, both the restraining matrix as well as the cellular domain extend to infinity. For the same reason, the equations obtained from the limiting values of the Eshelby tensor are not applicable to describe the behavior of the cellular domain in the limits of infinitely soft and infinitely rigid matrices (because the limit that defines the geometry is taken before the limiting value of the elastic constants). For those cases, as discussed in the text, one has to use the more general Eshelby tensor [44] for finite domains and take the corresponding limit of the elastic moduli.

We define the permittivity coefficients $\epsilon_s=\chi_s+1$ and $\epsilon_v=\chi_v+1$ [25]. In the orientational polarization, $\epsilon_v=1$, and in the axially induced mechanism, $\epsilon_s=\epsilon_v$; we thus find the following.

For free boundaries, $\mu_m\to 0$, $\kappa_m\to 0$,

$$P_{xx}^{self}=P_{zz}^{self}=0, \quad u_{xx}^{tot}=u_{zz}^{tot}=\frac{\rho p^0}{9\kappa_c}. \tag{B1}$$

For fixed boundaries, $\mu_m\to\infty$, $\kappa_m\to\infty$,

$$P_{xx}^{self}=P_{zz}^{self}=\frac{\rho p^0}{3}(\epsilon_v-1), \quad u_{xx}^{tot}=u_{zz}^{tot}=0. \tag{B2}$$

Otherwise, for disks,

$$P_{xx}^{self}=\frac{\rho p^0}{3}\frac{2\mu_c[\epsilon_s(3\epsilon_v-2)-\epsilon_v]}{3\epsilon_v\kappa_c+4\epsilon_s\mu_c},$$

$$P_{zz}^{self}=\frac{\rho p^0}{3}\frac{4\mu_c(\epsilon_v-\epsilon_s)}{3\epsilon_v\kappa_c+4\epsilon_s\mu_c},$$

$$P_{ii}^{self}=\frac{\rho p^0}{3}\frac{12\mu_c\epsilon_s(\epsilon_v-1)}{3\epsilon_v\kappa_c+4\epsilon_s\mu_c}, \tag{B3}$$

$$u_{xx}^{tot}=0, \quad u_{zz}^{tot}=\frac{\rho p^0\epsilon_v}{3\epsilon_v\kappa_c+4\epsilon_s\mu_c}; \tag{B4}$$

for spheres,

$$P_{xx}^{self}=P_{zz}^{self}=\frac{1}{3}P_{ii}^{self}=\frac{\rho p^0}{3}\frac{4\mu_m(\epsilon_v-1)}{3\epsilon_v\kappa_c+4\mu_m}, \tag{B5}$$

$$u_{xx}^{tot}=u_{zz}^{tot}=\frac{1}{3}u_{ii}^{tot}=\frac{\rho p^0}{3}\frac{\epsilon_v}{3\epsilon_v\kappa_c+4\mu_m}; \tag{B6}$$

for rods,

$$P_{xx}^{self}=\frac{\rho p^0}{3}\frac{3\mu_m(\epsilon_v-1)+\mu_c(\epsilon_v-\epsilon_s)}{3\kappa_c\epsilon_v+\mu_c\epsilon_s+3\mu_m},$$

$$P_{zz}^{self}=\frac{\rho p^0}{3}\frac{3\mu_m(\epsilon_v-1)+\mu_c[\epsilon_v(3\epsilon_s-2)-\epsilon_s]}{3\kappa_c\epsilon_v+\mu_c\epsilon_s+3\mu_m},$$

$$P_{ii}^{self}=\frac{\rho p^0}{3}\frac{3(\epsilon_v-1)(3\mu_m+\epsilon_s\mu_c)}{3\kappa_c\epsilon_v+\mu_c\epsilon_s+3\mu_m}, \tag{B7}$$

$$u_{xx}^{tot}=\frac{\rho p^0\epsilon_v}{2(3\kappa_c\epsilon_v+\mu_c\epsilon_s+3\mu_m)}, \quad u_{zz}^{tot}=0. \tag{B8}$$

[1] D. E. Ingber, Ann. Med. **35**, 1 (2003).
[2] C. S. Chen, J. Tan, and J. Tien, Annu. Rev. Biomed. Eng. **6**, 275 (2004).
[3] D. E. Discher, P. Janmey, and Y. Wang, Science **310**, 1139 (2005).
[4] I. B. Bischofs and U. S. Schwarz, Proc. Natl. Acad. Sci. U.S.A. **100**, 9274 (2003).
[5] I. B. Bischofs, S. A. Safran, and U. S. Schwarz, Phys. Rev. E **69**, 021911 (2004).
[6] C. H. K. Kurpinski, J. Chu, and S. Li, Proc. Natl. Acad. Sci. U.S.A. **103**, 16095 (2006).
[7] J. Folkman and H. P. Greenspan, Biochim. Biophys. Acta **417**, 211 (1975).
[8] S. Huang and D. E. Ingber, Nat. Cell Biol. **1**, E131 (1999).
[9] D. E. Ingber, Cancer Cells **8**, 175 (2005).
[10] B. Shraiman, Proc. Natl. Acad. Sci. U.S.A. **102**, 3318 (2005).
[11] C. M. Nelson, R. P. Jean, J. L. Tan, W. F. Liu, N. J. Sniadecki, A. A. Spector, and C. S. Chen, Proc. Natl. Acad. Sci. U.S.A. **102**, 11594 (2005).
[12] A. J. Engler, S. Sen, H. L. Sweeney, and D. E. Discher, Cell **126**, 677 (2006).
[13] And patterning in general.
[14] A. D. Bershadsky, N. Q. Balaban, and B. Geiger, Annu. Rev. Cell Dev. Biol. **19**, 677 (2003).
[15] D. Riveline, E. Zamir, N. Q. Balaban, U. S. Schwarz, T. Ishizaki, S. Narumiya, Z. Kam, B. Geiger, and A. D. Bershadsky, J. Cell Biol. **153**, 1175 (2001).
[16] A. Besser and S. A. Safran, Biophys. J. **90**, 3469 (2006).
[17] M. Eastwood, V. C. Mudera, D. A. McGrouther, and R. A. Brown, Cell Motil. Cytoskeleton **40**, 13 (1998).
[18] A. M. Collinsworth, C. E. Torgan, S. N. Nagda, R. J. Rajalingam, W. E. Kraus, and G. A. Truskey, Cell Tissue Res. **302**, 243 (2000).
[19] K. Takakuda and H. Miyairi, Biomaterials **17**, 1393 (1996).
[20] J. H. C. Wang, P. Goldschmidt-Clermont, J. Wille, and F. C. P. Yin, J. Biomech. **34**, 1563 (2001).
[21] R. Kaunas, P. Nguyen, S. Usami, and S. Chein, Proc. Natl. Acad. Sci. U.S.A. **102**, 15895 (2005).
[22] R. De, A. Zemel, and S. A. Safran, Nat. Phys. (to be published).
[23] F. Grinnell, Trends Cell Biol. **10**, 362 (2000).
[24] C. M. Lo, H. B. Wang, M. Dembo, and Y. L. Wang, Biophys. J. **79**, 144 (2000).
[25] A. Zemel, I. B. Bischofs, and S. A. Safran, Phys. Rev. Lett. **97**, 128103 (2006).
[26] K. Jakab, A. Neagu, V. Mironov, R. R. Markwald, and G. Forgacs, Proc. Natl. Acad. Sci. U.S.A. **101**, 2864 (2004).
[27] T. Korff and H. G. Augustin, J. Cell. Sci. **112**, 3249 (1999).
[28] C. Kittel, *Introduction to Solid State Physics*, 6th ed. (John Wiley and Sons, New York, 1986).
[29] G. Wei and S. F. Edwards, Phys. Rev. E **58**, 6173 (1998).
[30] In many experiments, the cells are placed in various types of gels; in some biological contexts, however, these gels may originate in the extracelluar matrix produced by the cell itself.
[31] The condition that the surroundings are cell-free is added for clarity of the model only. This is not a necessary requirement as long as the surroundings (like the cellular domain) are isotropic and elastic, and mechanically distinct from the cellular domain. That is, the cell type and/or the elastic matrix in the cellular domain differ from that of the surrounding material.

[32] Higher-order corrections (nonlinear terms) imply a dependence of the cell susceptibility $\boldsymbol{\chi}$ on the field u_{ij}. A number of experiments [3,21,45] have shown that when the elastic field due to the cells was augmented by an external field and the resultant field increased beyond some cell-specific, threshold value [3,45], the typical cellular response was reversed, and a lowering of the contractile force [45] as well as a perpendicular rather than a parallel alignment of the stress fibers [21] was observed; this implies a change in the sign of the susceptibility. A recent theoretical model has recently been presented that accounts for nonlinear effects of this sort and provides a mechanical explanation for the dependence of cell orientation on the magnitude of the matrix strain [46,47]. In addition, in a forthcoming paper, we show that this nonlinear behavior may also account for the unusual orientational response of cells in cyclically varying strain fields [22]. In this paper we focus on the linear response of the cells to static stresses and show that, even in this case, the cells can self-polarize under certain conditions.

[33] $$G_{ij} = \frac{a}{|\mathbf{r}-\mathbf{r}'|}\left[(3-4\nu)\delta_{ij} + \frac{(r_i - r_i')(r_j - r_j')}{|\mathbf{r}-\mathbf{r}'|^2}\right]$$

with $a=1/[16\pi\mu(1-\nu)]$.

[34] L. D. Landau and E. M. Lifshitz, *Theory of Elasticity*, 3rd ed., Course of Theoretical Physics Vol. 7 (Butterwoth-Heinemann, Oxford, 1999).

[35] J. D. Eshelby, Proc. R. Soc. London, Ser. A **241**, 376 (1957).

[36] For the resulting stress we may write $\sigma_{ij}^c = \rho \mathbf{CSC}^{-1}\langle p_{ij}\rangle$. The tensor $\mathbf{CSC}^{-1}$ that relates the polarization stress to the resulting stress in the cellular domain is the elastic equivalent of the depolarization factor in electrostatics.

[37] J. D. Eshelby, Proc. R. Soc. London, Ser. A **252**, 561 (1959).

[38] $I_{ijkl} = (\delta_{ik}\delta_{jl} + \delta_{il}\delta_{jk})/2$.

[39] T. Mura, *Micromechanics of Defects in Solids* (Kluwer Academic, Dordrecht, 1991).

[40] R. A. Brown, K. K. Sethi, I. Gwanmesia, D. Raemdonck, M. Eastwood, and V. Mudera, Exp. Cell Res. **274**, 310 (2002).

[41] In this paper, we do not consider plastic deformations of the extracellular matrix, which often occur by specialized cell activity (e.g., fibroblasts in collagen gel) [40].

[42] The elements of the Eshelby tensor are given as a function of the aspect ratio of spheroidal domains, for example, in [44].

[43] J. Fringer and F. Grinnell, J. Biol. Chem. **276**, 31047 (2001).

[44] T. S. Chow, J. Appl. Phys. **48**, 4072 (1977).

[45] R. A. Brown, R. Prajapati, D. A. McGrouther, I. V. Yannas, and M. Eastwood, J. Cell Physiol. **175**, 323 (1998).

[46] D. Stamenović, Mol. Cell Biomech. **2**, 69 (2005).

[47] K. A. Lazopoulos and D. Stamenović, Mol. Cell Biomech. **3**, 43 (2006).