Active Elasticity of Gels with Contractile Cells

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Cells play an active role in the maintenance of mechanical homeostasis within tissues and their response to elastic forces is important for tissue engineering. We predict the collective response of an ensemble of contractile cells in a three-dimensional elastic medium to externally applied strain fields. Motivated by experiment, we model the cells as polarizable force dipoles that change their orientation in response to the local elastic strain. The analogy between the mechanical response of these systems and the dielectric response of polar molecules is used to calculate the elastic response function. We use this analogy to evaluate the average cell orientation, the mean polarization stress, and the effective elastic constants of the material, as a function of the cell concentration and matrix properties.

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Mechanical forces acting externally on entire tissues, or generated internally by the contractile activity of individual cells within a tissue, play an important role in many physiological processes, such as bone and muscle growth, wound healing, angiogenesis, and the maintenance of mechanical homeostasis [1,2]. A fundamental understanding of the physical properties of these active processes is of prime importance in tissue engineering [1,3].

The responses of tissues to elastic forces are quite different from the passive mechanical properties of ''dead'' composite materials. On short time scales the passive elastic response of the matrix and the cellular cytoskeleton [4] dominate the mechanical response of the tissue; however, on longer time scales many cell types [such as muscle cells, fibroblasts, endothelial cells [1]] respond to applied forces by *actively* adjusting the cellular force pattern and its polarity. Cells embedded within three-dimensional hydrogels [5,6], or deposited on elastic substrates [7], were seen to polarize in the direction of a *static* strain field on a time scale between hours and days, and to return to their initial, isotropic state when the field was removed [8]. If the system is acted upon by a fixed load that tends to expand the material, the resulting enhancement of cellular contractile forces in the direction of the external stress diminishes the net strain in the medium; this results in an effectively more rigid and thus more stable system—in accord with the principle of homeostasis [1].

Elastic deformations caused by localized, cellular contractile forces give rise to long-range, elastic interactions between cells [10] that scale as $1/d^3$ where d is the intercellular distance. Previous theoretical models have highlighted the role played by cellular traction forces in modulating the mechanical nature of gels, but have not accounted for the mechanical consequences of the long-range elastic interactions between cells [11,12].

In this Letter, we present a quantitative theory that focuses on the (long-range) collective elastic response of an ensemble of active cells to *static* forces to predict the cell polarization and effective elastic constants of the system, as a function of the cell concentration and matrix properties. We use an analogy to the dielectric response of polar molecules to provide a simple mathematical framework for analyzing the polarization behavior of cells. Our focus is on the long-time orientational response of the cells and the active forces they exert; the effects we predict occur even if the (mean) elastic moduli of the cells and the medium are identical.

Macroscopic theory: cell polarization and effective elastic moduli.—In the absence of external forces, the composite system of cells and matrix is isotropic (and homogeneous on scales much larger than the cell size) since we consider materials in which the cells are uniformly distributed and randomly oriented. The addition of active cells to a gel results in a relatively short time-scale compression of the overall material due to the intrinsic contractile forces exerted by the cells [13]. The system then reaches a state of mechanical equilibrium in which the stress in the medium, σ_{ij}^0, is balanced by the forces exerted by the cells. We choose this as our reference state.

The excess strains, u_{ij}, and stresses, σ_{ij}, (relative to the reference state) in the system are related by the renormalized elastic moduli, $\mathbf{C}$, of the entire composite including both the cells and the matrix; $\sigma_{ij} = \mathbf{C}u_{ij}$ [14]. Our interest in this Letter is not in the derivation of the instantaneous response given by $\mathbf{C}$ of the gel-cell composite from the elastic properties of the individual cells and the matrix, and the interactions between them [cf. Ref. [12]], but rather to quantify the active changes that occur on longer time scales due to the collective polarization response of the cells in the presence of external fields [15].

The long-range elastic deformations caused by the contractile activity of each cell are dominated by the contribution due to the force dipole tensor, $p_{ij} = \sum f_i l_j$, where $\vec{f}$

and $\vec{l}$ are the force at, and the radius vector to, each adhesion contact of the cell with the matrix, and the sum is over all contacts [10]. The polarization of cells in response to applied forces involves a reassembly of the cellular force pattern that in turn, changes p_{ij}. The macroscopic measure of cell polarization is the (ensemble) mean, $\rho\langle p_{ij}\rangle$, where ρ is the number of cells per unit volume. The polarization of the cells in the direction of the principal strain direction can be written as follows:

$$P_{ij} = \rho(\langle p_{ij}\rangle - \langle p_{ij}\rangle_0) = -\boldsymbol{\chi}\sigma_{ij} = -\boldsymbol{\xi}u_{ij}. \qquad (1)$$

The polarization tensor, P_{ij}, measures the increase in the mean dipole tensor relative to its value in the absence of external forces and cell interactions, $\rho\langle p_{ij}\rangle_0 = \sigma^0_{ij}$ [see Eq. (7) below]. The forth-rank tensors $\boldsymbol{\chi}$ and $\boldsymbol{\xi}$ are, respectively, the susceptibility tensors for the excess stress and strain in the medium. These tensors contain all the effects of the elastic interactions among the cells that influence the orientational response of the cells to an applied field.

The total excess stress in the system is a superposition of the applied stress, σ^a_{ij}, and the polarization stresses due to cell activity: $\sigma_{ij} = \sigma^a_{ij} + P_{ij}$. This can be rewritten in terms of the susceptibility tensors as: $\sigma^a_{ij} = (\mathbf{I} + \boldsymbol{\chi})\sigma_{ij} = \boldsymbol{\epsilon}\sigma_{ij}$; $I_{ijkl} = \frac{1}{2}(\delta_{ik}\delta_{jl} + \delta_{il}\delta_{jk})$ is the fourth rank symmetric unit tensor. We find: $\sigma^a_{ij} = \tilde{\mathbf{C}}u_{ij}$ with $\tilde{\mathbf{C}} = \boldsymbol{\epsilon}\mathbf{C}$; or expressed in terms of the bulk and shear moduli as [16]:

$$\tilde{\kappa} = \epsilon_v\kappa \qquad \tilde{\mu} = \epsilon_s\mu. \qquad (2)$$

These effective elastic constants reflect the *active*, long-time, effective Hookean response of the system due to the response of the cell polarization to the applied stress. Our use of the elastic permittivity tensor, $\boldsymbol{\epsilon}$, allows us to exploit an analogy to the dielectric constant of polar materials; the quantity $\boldsymbol{\epsilon}$ reflects the collective, elastic dipolar screening effects that result in the entire system behaving as if it were more rigid, as explained below.

We next present a mean-field theory for calculating the permittivity tensor as function of cell (p, ρ) and matrix (κ, μ) parameters. Our model focuses on the long-range and active elastic interactions between the cells, and assumes that the cell concentration is below the critical value for phase transitions such as an isotropic to nematic transition as described by Gruler *et al.* [17], due to excluded volume interactions. Similarly to dielectrics, the permittivity tensor, $\boldsymbol{\epsilon}$, depends on the mechanism by which the cells polarize. In the following, we focus on systems of cells with bipolar morphologies (e.g., muscle cells and often fibroblasts) for which it has been shown that the sum of the forces exerted on adhesion contacts gives rise to two (approximately) equal and oppositely directed *contractile* forces centered on two opposite ends of the cell [18]. In this approximation, each cell is modeled by an anisotropic force dipole tensor: $p_{ij} = (fl)\hat{l}_i\hat{l}_j = p\hat{l}_i\hat{l}_j$, where $\vec{f}$ is the force, $\vec{l}$ is the dipole separation, and p is the dipole strength [typically $|p| \approx 10^{-11}$ J [19]]. For contraction dipoles $p < 0$. In the model presented below the magnitude of the force dipoles, p, is fixed but is free to vary in direction. This is appropriate to the case of cells where the adhesion contacts have saturated in size; however, one can also consider the more general case in which the dipole strength changes in response to the stress [20]. The simplification of fixed p has a simple (experimentally testable) consequence that external forces do not change the compressional response of the system, namely $\tilde{\kappa} = \kappa$ (or $\epsilon_v = 1$), see below.

Microscopic theory of the orientational elastic polarization.—To predict the susceptibilities from a microscopic model we must average the dipole tensor. We shall do this using an ensemble that is analogous to the Boltzmann ensemble for thermal systems; the determining factor in this ensemble is the elastic energy. It was recently shown [10,21] that the optimal orientation of cells in the presence of an elastic strain field can be predicted by minimizing the interaction energy of the cellular dipole, p_{ij}, with the local strain in the cell vicinity, u^{loc}_{ij}:

$$W = p_{ij}u^{\mathrm{loc}}_{ij}. \qquad (3)$$

To calculate the local stain field we adopt a mean-field formalism inspired by the original theory of polar dielectrics by Onsager [22] that was later proved to be exact to third order in the (electric) dipole density [23,24]. The approach we take is also very similar, but not identical, to the so-called self-consistent approach used for composite materials (e.g., Ref. [25]). Here, however, the local field is introduced to find a *statistical weight* for each cell orientation that may change due to the active biological response of the cells.

To calculate the local strain that acts on each cell in the ensemble, we consider an infinite medium that contains cells—with an average strain, u_{ij}, in the medium. We focus on one *central* cell in the system. The *local* field, u^{loc}_{ij}, that polarizes the cell differs from the average field, u_{ij}, because the latter includes the (as yet unknown) mean contribution of the central cell itself. Following Onsager, we make the simple approximation that the permittivity parameters ϵ_s and ϵ_v remain uniform in the surrounding matrix, but are equal to unity in a region from which the central cell has been (artificially) removed. This process reduces the problem to the determination of the field in a spherical inhomogeneity with the elastic constants of the "passive" (composite) matrix, $\mathbf{C}$, within an infinite medium characterized by the (as yet unknown) effective moduli, $\tilde{\mathbf{C}} = \boldsymbol{\epsilon}\mathbf{C}$ and subject to the strain field at infinity, u_{ij}. The solution to the elastic problem of a spherical inhomogeneity is given by the well-known formula [26]:

$$u^{\mathrm{loc}}_{ij} = \tfrac{1}{3}(a_v - a_s)u_{kk}\delta_{ij} + a_s u_{ij} \qquad (4)$$

with

$$a_v = \frac{\tilde{\kappa}}{\tilde{s}_v(\kappa - \tilde{\kappa}) + \tilde{\kappa}}, \qquad a_s = \frac{\tilde{\mu}}{\tilde{s}_s(\mu - \tilde{\mu}) + \tilde{\mu}}, \quad (5)$$

where $\tilde{s}_v = \frac{1}{3}(1 + \tilde{\nu})/(1 - \tilde{\nu})$ and $\tilde{s}_s = \frac{2}{15}(4 - 5\tilde{\nu})/(1 - \tilde{\nu})$; and where $\tilde{\nu}$ is the effective Poisson ratio.

In order to calculate the susceptibilities χ_v and χ_s we self-consistently calculate the elements of the mean dipole tensor, $\langle p_{ij} \rangle$, for the situation of simple stretching in which the mean stress *in the matrix* is a uniaxial tension, T, along the z axis, namely: $\sigma_{ij} = T\delta_{iz}\delta_{jz}$ (recall that the corresponding applied load is given by $\sigma^a_{ij} = \epsilon\sigma_{ij}$). Using Eq. (3) one finds [27]:

$$W = \frac{a_s pT}{2\mu}\cos^2\theta + \left(\frac{a_v}{9\kappa} - \frac{a_s}{6\mu}\right). \quad (6)$$

In order to calculate $\langle p_{ij} \rangle$ we must specify the probability distribution function. One approach to the problem is based on the observation discussed above that the elastic energy, W, determines the optimal cell orientation. This suggests the use of Boltzmann distribution: $\mathcal{P}(\Omega) \sim e^{-\lambda W(\Omega)}$, where Ω is the solid angle. The "noise" factor, λ, is a measure of the energy scale associated with the instantaneous stochastic assembly and disassembly of all the focal adhesions and stress fibers within each cell; this may be cell and matrix specific. Part of this energy is thermal in origin because the adsorption of proteins to these adhesions is governed by local equilibrium considerations. Similar use of the Boltzmann distribution was made in other studies of cellular systems, e.g., [3,9]. Kemkemer *et al.* [9] have shown that the Boltzmann-like distribution can account for the experimentally observed, orientational response of cells in a periodically varying stress field.

A similar distribution function for the cell polarization can be motivated by a model in which the direction of the dipole is determined from a competition between the cell alignment due to the external field, and due to a random force that results from the heterogeneity of the gel.

For simplicity, we use the Boltzmann distribution to derive an expansion of $\langle p_{ij} \rangle$ in powers of the tension, T:

$$\langle p_{zz} \rangle = \frac{p}{3} - \frac{2}{45}\frac{\lambda p^2 a_s}{\mu}T + \cdots \quad (7)$$

and the relation: $2\langle p_{xx} \rangle = 2\langle p_{yy} \rangle = p - \langle p_{zz} \rangle$. Off diagonal elements, $\langle p_{\alpha\beta} \rangle$, are zero. The first term in the expansion is the contraction stress in the reference state: $\sigma^0_{xx} = \sigma^0_{yy} = \sigma^0_{zz} = \frac{1}{3}\rho p$. The second terms in the expansion of $\langle p_{zz} \rangle$ and $\langle p_{xx} \rangle$ are susceptibility elements of the form $\chi_\parallel = \chi_{\alpha\alpha\alpha\alpha}$ and $\chi_\perp = \chi_{\alpha\alpha\beta\beta}$, respectively, that are related to χ_s and χ_v through: $\chi_s = \chi_\parallel - \chi_\perp$ and $\chi_v = \chi_\parallel + 2\chi_\perp$. We thus find:

$$\chi_s = \epsilon_s - 1 = \rho a_s(\epsilon_s)\alpha \qquad \chi_v = \epsilon_v - 1 = 0 \quad (8)$$

with $\alpha = \lambda p^2/(15\mu)$. We call this factor the orientational polarizability of the cell, in analogy to the orientational polarizability of polar molecules, $\beta p^2/3$, where β^{-1} is thermal energy.

The dependence of ϵ_s on ρ, α, and ν can be derived in a self-consistent manner as a cubic equation in ϵ_s from Eqs. (5) and (8). For low cell concentrations we find the following expansion of ϵ_s in powers of ρ:

$$\epsilon_s = 1 + \rho\alpha + \frac{2(4 - 5\nu)}{15(1 - \nu)}\rho^2\alpha^2 + \cdots. \quad (9)$$

Results.—The orientational response of the cellular force dipole to a tensile strain gives rise to screening effects that are similar to those of an electric dipole in a dielectric. The screening effect of an ensemble of elastic dipoles is reflected in an effective rigidification of the medium, namely, the strain field in response to applied forces is diminished by the polarization field of the cells.

Figure 1 summarizes the mechanical properties of the system as a function of cell concentration. The contribution of the cellular polarization to the long-time active elastic response of the medium, $\tilde{\mathbf{C}}$, is contained in the permittivity tensor $\epsilon = \tilde{\mathbf{C}}\mathbf{C}^{-1}$. The relative change in several effective moduli: the shear modulus, $\tilde{\mu}/\mu = \epsilon_s$, the bulk modulus, $\tilde{\kappa}/\kappa = \epsilon_v$, the Young's modulus, $\tilde{E}/E$, and the Poisson ratio, $\tilde{\nu}/\nu$, is plotted as a function of the dimensionless quantity, $\rho\alpha$, which is proportional to the cell concentration (ρ); recall that $\alpha \sim \lambda p^2/\mu$ has dimensions of volume and may be interpreted as the effective volume of influence of the cell. The left panel shows the behavior for $\nu = 0.3$ and the right panel is for $\nu \approx 0.5$.

The active forces exerted by the cells result in a rigidification of the material as evidenced by the increase of both the effective shear, $\tilde{\mu}$, and the effective Young's moduli, $\tilde{E}$, as shown in Fig. 1. The saturation of $\tilde{E}$ with increasing cell concentration reflects the fact that $\tilde{\kappa} = \kappa$ (see below), as evident from the relation $\tilde{E} = 9\tilde{\kappa}\tilde{\mu}/(3\tilde{\kappa} + \tilde{\mu})$. The latter result, however, should be taken with caution in view of the limited accuracy of the Onsager model at high concentrations due to higher order correlations, ex-

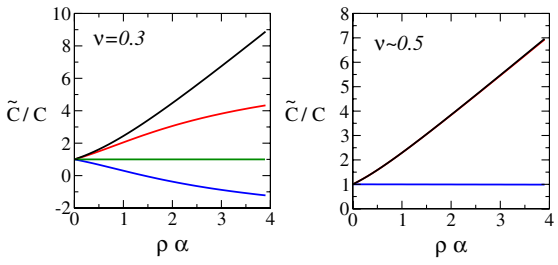

FIG. 1 (color online). The elastic permittivity constants and the *relative change* in the effective elastic moduli: Black—shear modulus, red—Young's modulus, blue—Poisson ratio, and green—bulk modulus. The black and green curves are (also) the two elastic permittivity constants, ϵ_s and ϵ_v, respectively. For incompressible materials (right panel), $\tilde{\nu} = \nu = 0.5$ and $\tilde{\mu}/\mu = \tilde{E}/E$.

cluded volume interactions, etc. The Poisson ratio, $\tilde{\nu}$, decreases monotonically with increasing cell concentration, attaining negative values (> -1) for high enough values of $\rho\alpha$. As the cells orient parallel to the applied uniaxial stretch, the overall contraction in the parallel direction increases while the contraction in the perpendicular direction decreases. This results in an overall net force pushing outward in the perpendicular directions that opposes the natural tendency of the matrix to contract laterally. This results in a smaller effective Poisson ratio for media that are not totally incompressible.

Our results that predict no change in the effective bulk modulus are applicable so long as there is no change in the magnitude of the elastic dipole moment of the cells due to the external force [20].

We also predict the dependence of the mean polarization, P_{ij} (of the cells in the bulk), on cell concentration in two simple situations: (i) The medium is uniaxially stretched along the z axis by a fixed load with free surfaces normal to the $\hat{x}$ and $\hat{y}$ directions: $\sigma^a_{zz} = T^a$ and $\sigma^a_{xx} = \sigma^a_{yy} = 0$. (ii) The medium is stretched with a constant strain in the $\hat{z}$ direction with fixed surfaces in both the $\hat{x}$ and $\hat{y}$ directions: $u_{zz} = \Delta l/l$ and $u_{xx} = u_{yy} = 0$. Note that in this case, the cellular force is balanced by equivalent forces that hold the boundaries: $\sigma^a_{zz} = (\tilde{\kappa} + \frac{4}{3}\tilde{\mu})\frac{\Delta l}{l}$ and $\sigma^a_{xx} = \sigma^a_{yy} = (\tilde{\kappa} - \frac{2}{3}\tilde{\mu})\frac{\Delta l}{l}$. In the analogous system of electrical dipoles, these two situations correspond, respectively, to a parallel plate capacitor with fixed charge, and to a capacitor with fixed voltage.

When the cell concentration is dilute ($\rho \to 0$), one expects that the mean force generated by the cells increases linearly with cell concentration; indeed we find: $P_{zz}(0) = -\frac{2}{3}\rho\alpha T^a$ in the first case and $P_{zz}(0) = -\frac{4}{3}\rho\alpha\mu\frac{\Delta l}{l}$ in the second case; in both cases $P_{xx} = P_{yy} = -P_{zz}/2 > 0$. The sign of the polarization elements shows that the average cellular force (developed relative to the reference state) points outward in the xy plane, and opposite to the external field in the $\hat{z}$ direction. Using Eqs. (5) and (8) we find that the normalized polarization, $P_{zz}/P_{zz}(0)$, is equal to a_s/ϵ_s in the first case and to a_s in the second case [27]. For fixed load, $P_{zz}/P_{zz}(0)$ decreases with ρ. This is because increasing the concentration of contractile cells results in a larger induced, contractile force that opposes the applied extensional stress, and diminishes the strain in the medium (indeed, $u_{zz} = T^a/\tilde{E}$ and $\tilde{E}$ increases with $\rho\alpha$). But the decrease in the local strain also decreases the tendency of the cells to align with the external stress. In contrast, if the strains $u_{zz} = \Delta l/l$ and $u_{xx} = u_{yy} = 0$ are held fixed, the elastic screening field is compensated by suitable tractions at the boundaries of the specimen. This results in a relative increase in the cell polarization as the cell concentration is increased.

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[16] $\epsilon_{ijkl} = \frac{1}{3}(\epsilon_v - \epsilon_s)\delta_{ij}\delta_{kl} + \frac{1}{2}\epsilon_s(\delta_{ik}\delta_{jl} + \delta_{il}\delta_{jk})$; an equivalent expression holds for χ_{ijkl}.
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