Fluidization of tissues by cell division and apoptosis

Jonas Ranft[a,b], Markus Basan[a], Jens Elgeti[a], Jean-François Joanny[a], Jacques Prost[a,c], and Frank Jülicher[b,1]

[a]Institut Curie, (Unité Mixte de Recherche 168, Institut Curie, Centre National de la Recherche Scientifique, Université Paris VI), 26 rue d'Ulm, 75248 Paris Cedex 05, France; [b]Max-Planck-Institut für Physik komplexer Systeme, Nöthnitzer Strasse 38, 01187 Dresden, Germany; and [c]Ecole Supérieure de Physique et de Chimie Industrielles, 10 rue Vauquelin, 75231 Paris Cedex 05, France

Edited by Tom C. Lubensky, University of Pennsylvania, Philadelphia, PA, and approved October 7, 2010 (received for review July 27, 2010)

During the formation of tissues, cells organize collectively by cell division and apoptosis. The multicellular dynamics of such systems is influenced by mechanical conditions and can give rise to cell rearrangements and movements. We develop a continuum description of tissue dynamics, which describes the stress distribution and the cell flow field on large scales. In the absence of division and apoptosis, we consider the tissue to behave as an elastic solid. Cell division and apoptosis introduce stress sources that, in general, are anisotropic. By combining cell number balance with dynamic equations for the stress source, we show that the tissue effectively behaves as a viscoelastic fluid with a relaxation time set by the rates of division and apoptosis. If the system is confined in a fixed volume, it reaches a homeostatic state in which division and apoptosis balance. In this state, cells undergo a diffusive random motion driven by the stochasticity of division and apoptosis. We calculate the expression for the effective diffusion coefficient as a function of the tissue parameters and compare our results concerning both diffusion and viscosity to simulations of multicellular systems using dissipative particle dynamics.

active fluids | fluctuations | growth processes | source stress

Many biological processes, such as organ development or cancerous tumor growth, involve the remodeling of tissues by cell division and cell death or apoptosis. For many years, emphasis has been put on the regulation of growth by signaling pathways such as growth factors and its genetic control (1, 2). Recently, however, the importance of the mechanical properties of tissues has been realized (3–7). It has been shown, for example, that during the development of the fruit fly *Drosophila*, the expression of some of the essential genes can be strongly modified by the application of external forces that change the local mechanical stresses acting on the cells in the growing organism (8). At certain stages of development, such as gastrulation, the spatial distribution of mechanical stresses also seems to play a role in controlling the pattern of gene expression (9). Quite similarly, in tumor progression, gene expression is related to the stress distribution in the tumor (7, 10).

The rates of cell division and cell death depend on many biological parameters, but they also depend on the local cell density or pressure in the tumor. It has recently been argued that the tissue pressure at which cell death exactly compensates cell division is an important parameter that could be related to the invasiveness of a tumor in a host tissue (11). Such a pressure has been called homeostatic pressure, and the corresponding tissue steady state, the homeostatic state. This pressure is defined as the isotropic part of the stress acting on cells directly and is not related in any simple way to the hydrostatic pressure.

From a mechanical point of view, a tissue is a complex system where the growth due to cell division and cell death interferes with the elastic deformation. Cell division and death often lead to unusual boundary conditions associated with both the fluxes of cells and local stresses. The mechanical properties of tissues have been described at various length scales. At the mesoscopic cellular scale, tissues have been described by analogy to foams as a network of cell junctions with a reorganization due to cell death and cell division (12, 13). This approach permits the consideration of key aspects of cellular behavior, but it usually is quasi-static and therefore does not capture the slow relaxation times of large wavelength modes. At a more macroscopic level, one can use a continuum mechanics or hydrodynamic approach (11, 14–16). In this approach, the tissue is described by macroscopic variables such as local deformations and local stresses, and a constitutive equation is required to study its mechanical properties. A recent review of the continuum description of growth processes emphasizing the role of nonlinear effects is found in ref. 17. In many cases, tissues can be considered as solids with linear or nonlinear elasticity that allows them to resist shear and compression (3, 18). The crumpling instabilities of plant tissues in leaves, for example, are very well accounted for by a description in terms of growing elastic materials (19). At higher shear stresses, tissues can yield and have been proposed to behave as plastic materials (20). Liquid-like behavior has, for example, been observed for embryonic tissues (21, 22). In this case, an elastic modulus is measured at short times and a viscosity at long times. The reported values of the shear modulus are of the same order as the shear modulus of the actin cytoskeleton in cells. The viscoelastic relaxation time of a tissue can be of the order of a few minutes (23). The question that we address here concerns the behavior of an elastic tissue on time scales long compared to that of cell division and apoptosis.

Reorganization processes such as the appearance of dislocation pairs in an ordered solid are known to relax elastic stresses only partly. Complete unbinding of dislocations is required to obtain full stress relaxation and melting (24). Even though division and apoptosis are clearly coupled to the tissue volume change and thus to the isotropic part of the stress, i.e., the tissue pressure, their coupling to the shear part of the stress is less obvious. Recent experiments done on single cells in a controlled environment such as patterned or deformable surfaces have clearly shown that one can orient the axis of cell division by applying an external constraint (25–27). Therefore, repeated rounds of cell division and apoptosis can affect both the isotropic and anisotropic parts of the tissue stress. The aim of this paper is to quantitatively study this effect in various situations.

This paper is organized as follows. In the next section, we consider tissues as elastic media and show that the coupling of cell division and cell death to the local stresses effectively leads to viscoelastic behavior with a relaxation time set by the rate of cell division. We first consider tissues in an isotropic homeostatic state. Our approach is then generalized to growing isotropic tissues and eventually to anisotropic tissue growth. In the subsequent section, we consider fluctuations of cell displacements and stresses in the tissue due to the stochasticity of cell division and calculate the diffusion constant of a tracer particle. The third section presents numerical simulations of dynamic tissues from which we determine both the diffusion constant of a cell and

Author contributions: J.R., M.B., J.E., J.-F.J., J.P., and F.J. performed research; and J.R., M.B., J.E., J.-F.J., J.P., and F.J. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]To whom correspondence should be addressed. E-mail: julicher@pks.mpg.de.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1011086107/-/DCSupplemental.

the tissue viscosity. The last section is devoted to a discussion of our results.

Growing Tissues as Elastic Media

We consider a tissue in which cells are linked to their neighbors by adhesion molecules. We assume that at short time scales this tissue behaves as an elastic solid. For small deformations, the tissue elasticity is described by a linear relation between stress and strain. For simplicity, we consider here only the case where the tissue is isotropic and described by a compressional modulus χ and a shear modulus μ.

At longer time scales, the tissue is remodeled by the appearance of new cells by division and the disappearance of cells by cell death. The cell number density ρ then obeys the balance equation

$$\partial_t \rho + \partial_\alpha(\rho v_\alpha) = (k_d - k_a)\rho, \qquad [1]$$

where $v_\alpha(\mathbf{r})$ is the cell velocity field at position $\mathbf{r}$, ∂_α denotes the partial derivative with respect to the coordinate r_α, and ∂_t is the partial time derivative. We use the Einstein convention and sum over repeated indices. The rates of cell division and apoptosis are denoted k_d and k_a, respectively.

Division and apoptosis imply a change of local stress generated actively. In a continuum description, the local stress associated with each event is a force dipole that can be described by a symmetric tensor $d_{\alpha\beta}$ because division and apoptosis do not generate any net torque. The associated force dipole density is $D_{\alpha\beta} = \sum_n d^{(n)}_{\alpha\beta}\delta(\mathbf{r} - \mathbf{r}_n)$. The elastic stress $\sigma^{\mathrm{el}}_{\alpha\beta}$ created by the force dipole density $D_{\alpha\beta}$ satisfies the force balance equation

$$\partial_\beta \sigma^{\mathrm{el}}_{\alpha\beta} = \partial_\beta D_{\alpha\beta}. \qquad [2]$$

The stress generated by cell division and apoptosis, $\sigma^s_{\alpha\beta} = -D_{\alpha\beta}$, acts as a source of stress in the tissue. The total stress $\sigma_{\alpha\beta} = \sigma^{\mathrm{el}}_{\alpha\beta} + \sigma^s_{\alpha\beta}$ satisfies the force balance $\partial_\beta \sigma_{\alpha\beta} = 0$. A detailed discussion of tissue stress force balance is presented in *SI Text*, *Force Dipoles in an Elastic Medium*.

For a simple elastic material without remodeling, the elastic stress is given by $\sigma^{\mathrm{el}}_{\alpha\beta} = C_{\alpha\beta\gamma\nu}u_{\gamma\nu}$, where the strain tensor $u_{\gamma\nu}$ describes the elastic deformation, and $C_{\alpha\beta\gamma\nu}$ is the tensor of elastic constants of the material. For an isotropic material, $C_{\alpha\beta\gamma\nu} = \chi\delta_{\alpha\beta}\delta_{\gamma\nu} + 2\mu(\delta_{\alpha\gamma}\delta_{\beta\nu} - \delta_{\alpha\beta}\delta_{\gamma\nu}/3)$. In the presence of cell division and apoptosis, a unique reference state of the strain can no longer be defined. However, differences of strain between subsequent states still have a meaning (see *SI Text*, *Dynamic Force Dipole Densities*), and we can write

$$\frac{\mathrm{D}}{\mathrm{D}t}\sigma_{\alpha\beta} = C_{\alpha\beta\gamma\nu}v_{\gamma\nu} + \frac{\mathrm{D}}{\mathrm{D}t}\sigma^s_{\alpha\beta}. \qquad [3]$$

Here, $v_{\alpha\beta} = (1/2)(\partial_\alpha v_\beta + \partial_\beta v_\alpha)$ is the strain rate tensor, $(\mathrm{D}/\mathrm{D}t)\sigma_{\alpha\beta} = \partial_t\sigma_{\alpha\beta} + v_\gamma\partial_\gamma\sigma_{\alpha\beta} + \omega_{\alpha\gamma}\sigma_{\gamma\beta} + \omega_{\beta\gamma}\sigma_{\alpha\gamma}$ denotes the convected corotational time derivative, and $\omega_{\alpha\beta} = (1/2)(\partial_\alpha v_\beta - \partial_\beta v_\alpha)$ is the vorticity of the flow. We introduce the isotropic and the traceless parts of the total stress, σ and $\tilde{\sigma}_{\alpha\beta}$, respectively, with $\sigma_{\alpha\beta} = \sigma\delta_{\alpha\beta} + \tilde{\sigma}_{\alpha\beta}$.

The rate of change of the isotropic component of the source stress is related to the rates of cell division and of apoptosis. Each cell division creates a positive isotropic contribution $d_d = d_{\alpha\alpha}/3 > 0$ to the isotropic force dipole $d_{\alpha\alpha}$ and each apoptosis event a negative contribution $d_a < 0$. Therefore, in a tissue the isotropic part of the source stress changes as

$$\frac{\mathrm{d}}{\mathrm{d}t}\sigma^s = -\rho(d_d k_d + d_a k_a), \qquad [4]$$

where $(\mathrm{d}/\mathrm{d}t) = \partial_t + v_\gamma\partial_\gamma$. Note that the rates of division and apoptosis k_d and k_a generally depend on local stress as well as on cell density. The isotropic part of the total stress then obeys

$$\frac{\mathrm{d}}{\mathrm{d}t}\sigma = \chi v_{\gamma\gamma} - \rho(d_d k_d + d_a k_a). \qquad [5]$$

We make here the assumption that the cell volume ρ^{-1} is under cellular control and depends on the isotropic part of the stress. In the simplest form, this assumption implies an equation of state $\sigma = \sigma(\rho)$ relating isotropic stress and cell density. As a consequence of this simple choice, σ depends only on the current cell configuration but not on history. Note that, in general, the relation between cell density and stress is more complex and can involve memory. The equation of state imposes that $\mathrm{d}\sigma/\mathrm{d}t = (\mathrm{d}\sigma/\mathrm{d}\rho)(\mathrm{d}\rho/\mathrm{d}t)$. Using Eq. **1** we find that this is compatible with Eq. **5** only if $\rho(d_d k_d + d_a k_a) = \chi(k_d - k_a)$, so that $d = d_d = -d_a$ and $d = \chi/\rho$. The total stress thus obeys

$$\frac{\mathrm{d}}{\mathrm{d}t}\sigma = -\frac{\chi}{\rho}\frac{\mathrm{d}\rho}{\mathrm{d}t}, \qquad [6]$$

which by using Eq. **1** can be rewritten as

$$\frac{\mathrm{d}}{\mathrm{d}t}\sigma = \chi[v_{\gamma\gamma} - (k_d - k_a)]. \qquad [7]$$

To discuss the traceless component of the source stress, the anisotropy of cells must be considered. This anisotropy determines the preferred axis of cell division and becomes apparent in the shape anisotropy of a given cell. It can be induced by external stresses or signaling cues, by internal factors, or by interactions between cells. Averaging this anisotropy in a small volume defines the nematic tensor $\tilde{q}_{\alpha\beta} = \langle n_\alpha n_\beta - \frac{1}{3}\delta_{\alpha\beta}\rangle$, where the unit vector $\mathbf{n}$ defines the axis of cell anisotropy. The rate of change of the nematic tensor to linear order is given by

$$\partial_t\tilde{q}_{\alpha\beta} = -\frac{1}{\tau_q}(\tilde{q}_{\alpha\beta} - \tilde{\sigma}_{\alpha\beta}/\sigma_0). \qquad [8]$$

Here we consider the case where the relaxation of the nematic tensor on a time scale $\tau_q > 0$ is driven mainly by the local anisotropic stress, and we ignore additional effects such as morphogen gradients. The response of the cell anisotropy to stress is described by the coefficient $\sigma_0 > 0$. Note that the isotropic component of the stress does not contribute to the relaxation of the nematic tensor, which is traceless. In the following, we consider the case where the anisotropy relaxation is faster than cell division and apoptosis such that $\tilde{q}_{\alpha\beta} \simeq \tilde{\sigma}_{\alpha\beta}/\sigma_0$.

Cell division is anisotropic. Each division event contributes a change $-\tilde{d}_{\alpha\beta}$ to the anisotropic component of the source stress $\tilde{\sigma}^s_{\alpha\beta}$. Because the cell division axis is on average aligned with the local tissue anisotropy, the force dipole $\tilde{d}_{\alpha\beta}$ is proportional to the nematic tensor: $\tilde{d}_{\alpha\beta} = \tilde{d}_d\tilde{q}_{\alpha\beta}$. The rate of change of the traceless part of the source stress is then given by

$$\frac{\mathrm{D}}{\mathrm{D}t}\tilde{\sigma}^s_{\alpha\beta} = -\rho(\tilde{d}_d k_d + \tilde{d}_a k_a)\tilde{q}_{\alpha\beta}. \qquad [9]$$

Here, we have added the contribution of force dipoles associated with apoptosis events $\tilde{d}_a$. Typically, $\tilde{d}_d > 0$ and $\tilde{d}_a < 0$. Using $\tilde{q}_{\alpha\beta} \simeq \tilde{\sigma}_{\alpha\beta}/\sigma_0$, we find that the total traceless stress obeys the constitutive relation

$$\left(1 + \tau_a\frac{\mathrm{D}}{\mathrm{D}t}\right)\tilde{\sigma}_{\alpha\beta} = 2\eta\tilde{v}_{\alpha\beta}, \qquad [10]$$

which corresponds to a Maxwell model of a viscoelastic material. The shear viscosity is $\eta = \tau_a\mu$ with a relaxation time $\tau_a^{-1} = \rho(\tilde{d}_d k_d + \tilde{d}_a k_a)/\sigma_0$. The Maxwell model implies that for long

times the traceless stress relaxes to zero and the tissue has a fluid behavior. This fact is a key result of this work.

Tissue elasticity together with the rate of change of the source stress define the properties of the tissue considered as an active material. In the following, we use this framework to discuss tissue behavior in various stationary and growing states.

Isotropic Homeostatic State. The isotropic homeostatic state is a homogeneous stationary state in which the cell density is constant ($\rho = \rho_h$), there is no cell flow ($v_\alpha = 0$), the nematic tensor vanishes ($\tilde{q}_{\alpha\beta} = 0$), and the source stress $\sigma^s_{\alpha\beta}$ is isotropic and time-independent. These conditions require that $k_d = k_a$ and $d_d k_d + d_a k_a = 0$. Because $d_d = -d_a = d$, both conditions are identical. Note that, strictly speaking, this is only true on average. The existence of an equation of state implies that $d\rho = \chi$. The condition $k_d(\rho) = k_a(\rho)$ determines the homeostatic density ρ_h and via the equation of state the isotropic stress $\sigma = \sigma(\rho_h) = -P_h$ (where P_h is the homeostatic pressure in the tissue).

Close to the homeostatic state, the properties of the tissue are obtained by expanding the effective cell number growth rate $k_d - k_a$ to linear order in the density deviations $\delta\rho = \rho - \rho_h$, and we write $k_d - k_a \simeq -\tau^{-1}\delta\rho/\rho_h$. Density deviations and stress deviations $\delta\sigma = \sigma + P_h$ are related via the equation of state. Thus, the tissue is described by (with $\zeta = \tau\chi$)

$$\left(1 + \tau\frac{\mathrm{d}}{\mathrm{d}t}\right)\delta\rho = -\rho_h \tau v_{\gamma\gamma}, \qquad \left(1 + \tau\frac{\mathrm{d}}{\mathrm{d}t}\right)\delta\sigma = \zeta v_{\gamma\gamma},$$
$$\left(1 + \tau_a\frac{\mathrm{D}}{\mathrm{D}t}\right)\tilde{\sigma}_{\alpha\beta} = 2\eta\tilde{v}_{\alpha\beta}. \qquad [11]$$

The first two equations are equivalent and show that the density and the isotropic part of the stress tend to relax to a fixed homeostatic density and pressure within a relaxation time τ with a Maxwell dynamics. The relaxation of the isotropic stress is the second central result of this work. Maxwellian dynamics of the isotropic part of the stress is a unique feature of the homeostatic state, which is absent in fluids with a conserved number of particles even at a liquid vapor critical point (28). This property is associated with the fact that, in the homeostatic state, the tissue is infinitely compressible. The pressure does not depend on the volume of the tissue because the number of cells is regulated by cell division and apoptosis. As a consequence, one can expect giant fluctuations of the volume of the tissue at constant (homeostatic) pressure. In a similar vein, the traceless part of the stress relaxes to zero with a relaxation time τ_a proportional to the cell division time k_d^{-1}. This stress relaxation under the influence of elastic dipole densities is a specific feature of tissues.

Growing Tissue. If the external pressure P^{ext} is different from the homeostatic pressure P_h, no homeostatic state exists. In this case, the isotropic stress does not relax, but the anisotropic stress still relaxes to zero in the absence of external anisotropic stress. Because shear stresses relax, the tissue is effectively viscoelastic and can still be described by a Maxwell model. The constitutive equations then read

$$\frac{\mathrm{d}}{\mathrm{d}t}\sigma = \chi[v_{\gamma\gamma} - \kappa(\rho)], \qquad [12]$$

$$\left(1 + \tau_a\frac{\mathrm{D}}{\mathrm{D}t}\right)\tilde{\sigma}_{\alpha\beta} = 2\eta\tilde{v}_{\alpha\beta}, \qquad [13]$$

where $\kappa(\rho) = k_d - k_a$ and $\sigma = \sigma(\rho)$.

A state of stationary growth with constant pressure and constant density exists with $\sigma(\rho) = -P^{\mathrm{ext}}$ and

$$v_{\gamma\gamma} = \kappa(\sigma), \qquad [14]$$

$$\tilde{\sigma}_{\alpha\beta} = 2\eta\tilde{v}_{\alpha\beta}. \qquad [15]$$

Stationary growth implies that the divergence of the velocity is constant, and in steady state (beyond the shear relaxation time τ_a) the tissue behaves as a viscous fluid under shear. In a spatially homogeneous system, the volume growth rate is $\kappa = k_d - k_a$. If the tissue is considered as incompressible, χ becomes large.

In a situation of isotropic growth of a system with spherical symmetry, the velocity field $\mathbf{v}$ of the cells can be calculated directly by using spherical coordinates, and we obtain $\mathbf{v} = (\kappa/3)\mathbf{r}$. The radius of the tissue is given by $\partial_t R(t) = v(R)$ and thus grows exponentially: $R(t) = R_0 e^{\kappa t/3}$.

Anisotropic Growth. In many situations, the growth of a tissue is anisotropic. Anisotropy arises because of tissue polarity where cell polarity in the tissue is aligned on large scales and characterized by the unit vector p_α. Such large-scale patterns of cell polarity are known to exist in epithelia and other tissues (29, 30). They could arise because of the existence of signaling gradients in the tissue, e.g., morphogen gradients, or could be because of cells aligning their polarity with their neighbors. Cell division is then on average oriented along the axis of cell polarity. Anisotropic stresses are generated by cell division, and thus growth is anisotropic.

If the anisotropy is set by an external field such as a morphogen gradient, the anisotropy of the tissue in the absence of stress is given by a traceless nematic tensor $\tilde{q}^0_{\alpha\beta}$. In the presence of stress, the nematic tensor in the tissue relaxes according to

$$\partial_t\tilde{q}_{\alpha\beta} = -\frac{1}{\tau_q}\left((\tilde{q}_{\alpha\beta} - \tilde{q}^0_{\alpha\beta}) - \frac{\tilde{\sigma}_{\alpha\beta}}{\sigma_0}\right). \qquad [16]$$

As in nematic elastomers, the elastic stress in the tissue depends both on the local deformation and on the order parameter. The traceless part of the elastic stress is given by

$$\tilde{\sigma}^{\mathrm{el}}_{\alpha\beta} = 2\mu\tilde{u}_{\alpha\beta} + w\tilde{q}_{\alpha\beta}, \qquad [17]$$

where w is the elasto-nematic coupling coefficient and we have ignored the elastic anisotropy for the sake of simplicity. The isotropic component of the elastic stress is still given by $\sigma^{\mathrm{el}} = \chi u_{\gamma\gamma}$. The source stress due to cell division and cell apoptosis is given by Eqs. **4** and **9**, as for an isotropic tissue, but the division and apoptosis rates become functions of the local density as well as of the local order parameter.

We now discuss the traceless component of the stress tensor. As for isotropic tissues, we assume that the orientation dynamics of the nematic tensor is fast compared to cell division. Using $\tilde{q}_{\alpha\beta} = \tilde{q}^0_{\alpha\beta} + \tilde{\sigma}_{\alpha\beta}/\sigma_0$, we find that the traceless part of the stress tensor satisfies

$$\left(1 + \tau_a\frac{\mathrm{D}}{\mathrm{D}t}\right)\tilde{\sigma}_{\alpha\beta} = 2\eta\tilde{v}_{\alpha\beta} - \sigma_0\tilde{q}^0_{\alpha\beta}. \qquad [18]$$

This equation is similar to the constitutive equation obtained for active polar gels as a description of the cell cytoskeleton (31). The stress relaxes over a time τ_a, and an anisotropic tissue therefore behaves as a Maxwell viscoelastic fluid. There is an additional component of the stress on the right-hand side of Eq. **18**, which is proportional to the spontaneous nematic tensor $\tilde{q}^0_{\alpha\beta}$. This stress has the same form as the active stress of ref. 31. Note that this active stress has a contribution proportional to the cell division rate and a contribution proportional to the apoptosis rate. The magnitude $-\sigma_0$ of the active stress is negative if the cells orient along the principal axis of the stress as the tissue grows. This situation corresponds to a dilative active stress. Active stresses in tissues have been first introduced by Bittig et al. (16).

An anisotropic tissue can reach a steady homeostatic stress. In the homeostatic state, the rates of cell death and cell division must be equal so that $k_d(\rho_h,\tilde{q}^0_{\alpha\beta}) = k_a(\rho_h,\tilde{q}^0_{\alpha\beta})$. The steady state behavior also implies that, after a round of cell division and cell death, the tissue goes back to the same mechanical state. This constraint imposes $\tilde{d}_d = -\tilde{d}_a$.

Let us first discuss this homeostatic state in the case of uniaxial order. In this case, one can write $\tilde{q}_{\alpha\beta} = q(p_\alpha p_\beta - \frac{1}{3}\delta_{\alpha\beta})$, where q is a measure of the degree of cell orientational order and p_α defines the macroscopic tissue polarity. Rotational invariance requires that both the duplication and apoptosis rates depend only on ρ and q but not on the direction of p_α. The existence of a tissue equation of state implies that $q = s(\rho)$ is a function of cell density ρ and that eventually the rates k_d and k_a are functions of ρ only. The steady state condition is now similar to that of isotropic liquids $k_d(\rho_h) = k_a(\rho_h)$, but this equality defines both ρ_h and q_h. As a consequence, if one measures the stress developed by a uniaxial tissue at steady state, the homeostatic stress in the symmetry axis direction is different from the homeostatic stress in the directions perpendicular to it. Conversely, in an ensemble where one imposes stresses, in order to obtain a homeostatic state one has to set both stresses to their homeostatic values. The case of biaxial order follows the same logic: There are two measures of the order independent of axis orientation (32), which also obey equations of state, and the steady state condition together with the two order parameters defines a homeostatic density. In this case, the tissue develops three different homeostatic stress values in three orthogonal directions of space. In turn, in order to obtain a steady state in a stress-imposed ensemble, one has to impose three different values in the three directions.

Fluctuations in a Homeostatic Tissue

In this section, we study the effect of noise on the mechanical properties of a tissue. For the sake of simplicity, here we consider only the vicinity of the homeostatic state of an isotropic nonpolarized tissue.

Density and Velocity Fluctuations. Cell division and apoptosis are stochastic processes. This stochasticity introduces noise in the cell number balance equation (Eq. **11**), which we now write as

$$\frac{\mathrm{d}}{\mathrm{d}t}\delta\rho + \rho_h v_{\gamma\gamma} = -\tau^{-1}\delta\rho + \xi_c. \qquad [19]$$

The cell division and apoptosis noise has a vanishing average $\langle\xi_c\rangle = 0$. Its correlation function can be approximated by writing the master equation for the number of cells in the absence of cell flow and assuming constant rates, leading to $\langle\xi_c(\mathbf{r},t)\xi_c(\mathbf{r}_0,t_0)\rangle = \rho_h(k_d + k_a)\delta(\mathbf{r}-\mathbf{r}_0)\delta(t-t_0)$. The isotropic stress fluctuation is related to the density fluctuation by the equation of state $\mathrm{d}\delta\sigma/\mathrm{d}t = -\chi/\rho_h \mathrm{d}\delta\rho/\mathrm{d}t$. Noise must also be introduced in the equation for the traceless part of the stress tensor, associated with fluctuations of cell shape and of the orientation of cell division, and thus we write

$$\left(1 + \tau_a \frac{\mathrm{D}}{\mathrm{D}t}\right)\tilde{\sigma}_{\alpha\beta} = 2\eta\tilde{v}_{\alpha\beta} - \tilde{\xi}_{\alpha\beta}. \qquad [20]$$

We do not give here a microscopic description of this noise. We assume only that the fluctuations are correlated over time scales much shorter than the cell division time so that this noise can be considered as local in time. It has zero mean, $\langle\tilde{\xi}_{\alpha\beta}\rangle = 0$, and because of the symmetry of the traceless component of the stress tensor its correlations are characterized by a noise strength θ with $\langle\tilde{\xi}_{\alpha\beta}(\mathbf{r},t)\tilde{\xi}_{\gamma\delta}(\mathbf{r}_0,t_0)\rangle = \theta[\delta_{\alpha\gamma}\delta_{\beta\delta} + \delta_{\alpha\delta}\delta_{\beta\gamma} - (2/3)\delta_{\alpha\beta}\delta_{\gamma\delta}]\delta(\mathbf{r}-\mathbf{r}_0)\delta(t-t_0)$.

We decompose all quantities in Fourier modes in space and time with the convention $f(\mathbf{q},\omega) = \int\mathrm{d}t\int\mathrm{d}\mathbf{r}e^{-i(\mathbf{q}\mathbf{r}-\omega t)}f(\mathbf{r},t)$. Using the force balance equation $\partial_\alpha\sigma_{\alpha\beta} = 0$ and Eqs. **19**, **20**, one can calculate the density fluctuation and the velocity fluctuation as a function of noise. The density fluctuation in the homeostatic state reads

$$\delta\rho = \frac{\tau\rho_h}{(1-i\omega\tau_a)\zeta + (1-i\omega\tau)\frac{4}{3}\eta}\left[\frac{4}{3}\eta\rho_h^{-1}\xi_c - \frac{q_\alpha\tilde{\xi}_{\alpha\beta}q_\beta}{q^2}\right], \qquad [21]$$

where $\zeta = \tau\chi$ is the effective bulk viscosity. In order to calculate the velocity fluctuation, one decomposes it into a longitudinal and a transverse component, $v_\alpha = v_{||}q_\alpha/q + v_{\perp\alpha}$, and we obtain

$$\begin{aligned} v_{||} &= \frac{1}{iq}\frac{1}{(1-i\omega\tau_a)\zeta + (1-i\omega\tau)\frac{4}{3}\eta}\left[(1-i\omega\tau_a)\zeta\rho_h^{-1}\xi_c\right. \\ &\quad \left. + (1-i\omega\tau)\frac{q_\alpha\tilde{\xi}_{\alpha\beta}q_\beta}{q^2}\right], \qquad [22] \\ v_{\perp\alpha} &= \frac{i}{\eta q^2}[\tilde{\xi}_{\alpha\beta}q_\beta - q_\alpha q_\gamma\tilde{\xi}_{\gamma\beta}q_\beta/q^2]. \end{aligned}$$

Diffusion of a Tracer Particle. In order to illustrate the role of the fluctuations in the tissue, we consider a tracer particle of radius a immersed in the tissue and moving by Brownian-type motion with the cell flow (33). If the particle follows the local velocity field in the tissue, its diffusion constant is given by

$$D = \frac{1}{3}\int_0^\infty \mathrm{d}\tau \int \frac{\mathrm{d}^3q}{(2\pi)^3}\langle e^{i\mathbf{q}[\mathbf{r}_\mathrm{p}(\tau)-\mathbf{r}_\mathrm{p}(0)]}\mathbf{v}(\mathbf{q},\tau)\mathbf{v}(-\mathbf{q},0)\rangle. \qquad [23]$$

We use the approximation that fluctuations in particle positions and velocity fluctuations in the tissue are decoupled and write the diffusion constant as $D = \int\frac{\mathrm{d}^3q}{3(2\pi)^3}\langle\mathbf{v}(\mathbf{q},\omega)\mathbf{v}(-\mathbf{q},-\omega)\rangle|_{\omega=0}$. The velocity correlation function can be directly calculated from Eq. **22**. The integral over the wave vector requires a maximum cutoff associated with the finite size of the particle $q_\mathrm{max} = 2\pi/(2a)$. The diffusion constant in the homeostatic state then reads

$$D = \frac{1}{3\pi a}\left\{\frac{1}{(\zeta+\frac{4}{3}\eta)^2}\left[\frac{\zeta^2 k_d}{\rho_h} + \frac{2}{3}\theta\right] + \frac{\theta}{\eta^2}\right\}. \qquad [24]$$

The diffusion constant therefore varies with the cell division rate k_d. In order to make the result more transparent, we assume in the following that the tissue is hardly compressible so that $\zeta \gg \eta$. In this limit, the expression for the diffusion constant reduces to $D = \frac{1}{3\pi a}[\frac{k_d}{\rho_h} + \theta(\frac{\rho_h\tilde{d}k_d}{\mu})^2]$. Here, we have expressed $\eta = \tau_a\mu$ and $\tilde{d} = \tilde{d}_d + \tilde{d}_a$. The diffusion coefficient increases with the cell division rate and varies linearly at small values of k_d. Note, however, that the noise intensity θ could itself be a function of k_d.

Numerical Simulations

In order to test the ideas presented in the previous sections, we perform numerical simulations of dynamic tissues. From these simulations we determine both the tissue viscosity and the diffusion constant of individual cells (which can be considered as tracer particles) as a function of the cell division rate in the homeostatic state.

Tissue Simulations. The procedure used to simulate tissues is detailed in *SI Text* (*Numerical Simulations*). In short, we use a few intuitive rules for cell behavior to simulate the growth of a three-dimensional tissue. Each cell is represented by two point particles that interact via a repulsive potential. The separation of the particles due to repulsion corresponds to cell growth. When the particles reach a critical distance, the cell divides. Cell division is described by inserting two new particles close to the initial

ones, thereby adding one new cell. As this process repeats, the tissue grows (see Fig. 1).

Neighboring cells interact with each other via a short-range repulsive and a long-range attractive potential. Furthermore, dissipative particle dynamics (DPD) (34, 35) is used to describe effects of internal friction and fluctuations. The DPD method can locally conserve momentum as required for hydrodynamic behavior. Finally, to mimic apoptosis, cells are randomly removed at a rate independent of time and pressure. An example of growth described by this model is provided in Movie S1.

Viscosity. In our simulations, we measure the shear viscosity of a tissue grown between two walls until reaching the homeostatic state (by using periodic boundary conditions in the plane). We shear the tissue by moving the top wall with a prescribed velocity relative to the bottom wall while keeping their distance fixed. The boundary conditions at the wall are described in *SI Text, Boundary conditions and measurement procedures*. We determine the shear rate from the measured velocity profile in the tissue, and we measure the stress exerted on the walls.

We first perform these simulations for a reference system with parameters given in *SI Text, Standard tissue and units*, and Table S1. Parameters used in the other simulations are specified relative to this reference system. For most values of the imposed velocity gradient, the stress is a nonlinear function of shear rate and the tissue shows shear thinning. At small shear rates, however, the viscosity is given by the ratio of stress to shear rate in linear response. Fig. 2 shows the variation of the viscosity η as a function of the division rate k_d for different parameter choices. For large values of k_d, the viscosity decreases as a function of the division rate with a power law $\eta \propto k_d^{-1.2}$ close to our prediction $\eta \propto k_d^{-1}$. Nonlinearities that are at the origin of the nonlinear rheology observed in our simulations are introduced in *SI Text, Nonlinearities*. Shear thinning will be discussed in a future publication.

On short time scales, the tissue behaves as a solid. However, if the imposed stress is larger than a critical yield stress, the tissue starts to flow.

Diffusion. In order to determine the diffusion coefficient of cells in the simulations, we let a tissue grow in a cubic compartment with periodic boundary conditions until it reaches the homeostatic state. Subsequently, we track cells individually and calculate their mean squared displacement (MSD). In the absence of cell turnover (no division or apoptosis), the displacement increases initially with time but saturates at a finite value. This saturation indicates caging and solid-like behavior. In the presence of cell division and apoptosis, the displacement shows diffusive behavior. We determine the diffusion coefficient by a linear fit to the MSD. The resulting diffusion coefficients displayed in Fig. 3 are proportional to k_d as expected from our theory. Note that the MSD of a cell over its lifetime remains the same for different values of the cell division rate.

Discussion

The first important result of this work is that cell division and apoptosis introduce a dynamic reorganization of elastic tissues

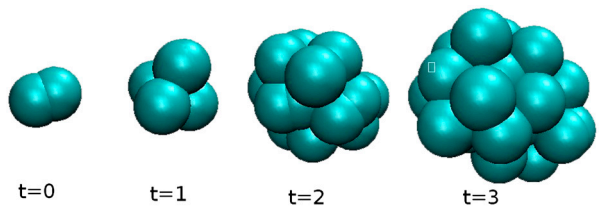


Fig. 1. Tissue growth simulation. The figure shows the first cell division and the early stages of tissue growth. Each cell is represented by two spheres.

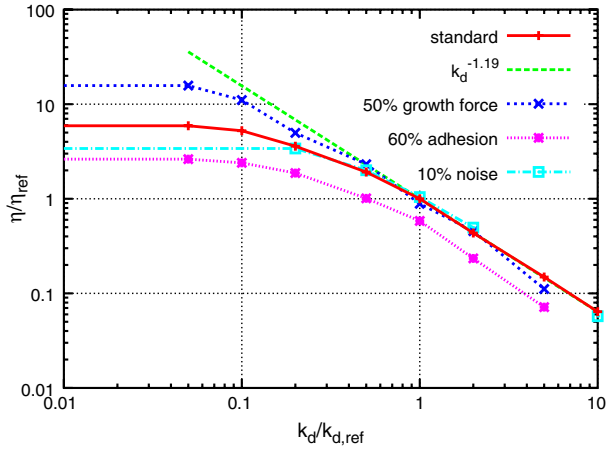


Fig. 2. Viscosity in a shear simulation. The viscosity is determined for different tissue parameters: standard/reference tissue (red), reduced growth force ($B^* = 0.5$, blue), reduced adhesion ($f_1^* = 0.6$, pink), and reduced noise intensity ($T_{noise}^* = 0.1$, light blue). The viscosity is rescaled to the value obtained for the reference tissue with reference cell division or apoptosis rate. We use simulation units p_0, t_0 and l_0; see *SI Text*. In these units, the viscosity of the standard tissue is $\eta_{ref} = 0.15 p_0 t_0$. The asterisks indicate dimensionless parameters that take the value 1 for the standard tissue (Table S1).

that leads to liquid-like behavior with well-defined shear and bulk viscosities on long time scales and in the vicinity of the homeostatic state. A unique consequence of these cellular reorganizations in the vicinity of the homeostatic state is the absence of a compression modulus. As a result, imposing cell pressures either slightly larger or slightly smaller than the homeostatic pressure leads either to the complete disappearance of the tissue or, on the contrary, to a complete invasion of space by the growing tissue. Our analytical calculation of the shear viscosity can well describe simulations capturing the essence of cell duplication and apoptosis. The relaxation of the stress in the tissue is because of a bias of the axis of cell rearrangements by local stress. Such a bias on the axis of cell division has been demonstrated in spectacular experiments for single cells in elastic environments (27, 36). However, it does not exist for all cell types. Whether or not these effects can be observed in practical situations depends on the actual values of τ and τ_a compared to the observa-

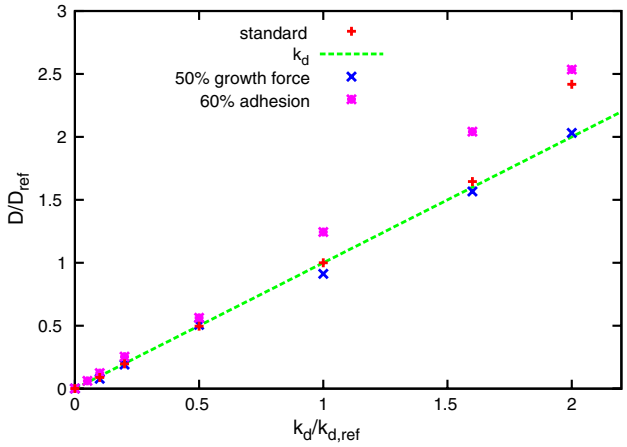


Fig. 3. Diffusion coefficient of cells D in a tissue simulation. Here, the dependence of D on the division rate k_d in the homeostatic state is shown for a reference system (red), simulations with reduced growth force ($B^* = 0.5$, blue), and decreased adhesion strength ($f_1^* = 0.6$, purple). The green line shows a linear fit to the data. The diffusion coefficient is rescaled to the value obtained for the reference tissue with reference cell division or apoptosis rate. In simulation units (see Table S1), $D_{ref} = 0.85 l_0^2 t_0^{-1}$.

tion time. For instance, in plants these times appear to be much longer than in many animal tissues.

The second important result of our work concerns the study of noise in the tissue. Here, we mostly considered the noise due to cell division and cell death. Other sources of noise such as the noise due to cell shape fluctuations (formation of protrusions for example) could also play an important role (37). Density correlation functions can be measured in the simulations and could be directly compared to experiments. In the future, such a fluctuation analysis could become an important way to characterize tissues. A spectacular illustration of the role of noise could be obtained in experiments in which a tissue is confined by a piston with a constant pressure equal to the homeostatic pressure acting on the tissue. Starting from the conservation equations with noise, one can easily show that the position of the piston is diffusing with a diffusion constant $D \simeq L(k_d + k_a)/(\rho S)$, where S is the area of the piston and L is the tissue thickness. These giant fluctuations are associated with the vanishing compressibility of the tissue that we obtain in the hydrodynamic theory.

In this study, we assumed that the only stress relaxation mechanisms are cell division and cell death. In the case where the adhesion is not too strong, other stress relaxation mechanisms can exist, for example, those related to fluctuations of cell shape. In this case, our predictions remain very similar, but the stress relaxation rate becomes the sum of the relaxation rates of the various relaxation modes. The viscous relaxation time can become much smaller than the cell division time, and accordingly the shear viscosity is strongly reduced. This smaller relaxation time is consistent with recent experiments on young tissues during development or on cancerous tissues where viscosities of the order of 10^5 Pa·s and viscoelastic relaxation times of the order of a few minutes have been measured (23, 38). However, these relaxation modes do not couple to the isotropic part of the stress, and the time scales for the compression and dilation deformations are still controlled by cell division and death.

In this paper, we presented only a linear description of the rheology of tissues. Some recent experiments suggest that tissues show shear thinning, i.e., that their viscosity decreases with the shear rate. This effect is also observed in our simulations when the shear rate is large compared to the division and apoptosis rates. Another nonlinear effect observed in the simulation is the existence of a yield stress that corresponds to a plastic behavior of the tissue. The yield stress again exists only at very low values of the cell division rate.

Last, we considered here that the tissue is a one-component fluid. We therefore implicitly neglect the roles of both the interstitial fluid and of the extracellular matrix, and we do not keep track of total mass conservation. Our approach can be generalized to take into account the regulation of cell division and cell death by growth factors and also the possible effects of the tissue mechanics on this regulation.

1. Wolpert L (2005) *Principles of Development* (Oxford Univ Press, Oxford), 2nd Ed.
2. Weinberg RA (2006) *The Biology of Cancer* (Garland Science, New York).
3. Fung YC (1993) *Biomechanics* (Springer, New York).
4. Shraiman BI (2005) Mechanical feedback as a possible regulator of tissue growth. *Proc Natl Acad Sci USA* 102:3318–3323.
5. Volokh KY (2006) Stresses in growing soft tissues. *Acta Biomater* 2:493–504.
6. Mammoto T, Ingber DE (2010) Mechanical control of tissue and organ development. *Development* 137:1407–1420.
7. Butcher DT, Alliston T, Weaver VM (2009) A tense situation: Forcing tumour progression. *Nat Rev Cancer* 9:108–122.
8. Farge E (2003) Mechanical induction of twist in the drosophila foregut/stomodeal primordium. *Curr Biol* 13:1365–1377.
9. Pouille PA, Ahmadi P, Brunet AC, Farge E (2009) Mechanical signals trigger myosin ii redistribution and mesoderm invagination in drosophila embryos. *Sci Signal* 2:ra16.
10. Paszek MJ, et al. (2005) Tensional homeostasis and the malignant phenotype. *Cancer Cell* 8:241–254.
11. Basan M, Risler T, Joanny JF, Sastre-Garau X, Prost J (2009) Homeostatic competition drives tumor growth and metastasis nucleation. *HFSP J* 3:265–272.
12. Farhadifar R, Röper JC, Aigouy B, Eaton S, Jülicher F (2007) The influence of cell mechanics, cell-cell interactions, and proliferation on epithelial packing. *Curr Biol* 17:2095–2104.
13. Hufnagel L, Teleman AA, Rouault H, Cohen SM, Shraiman BI (2007) On the mechanism of wing size determination in fly development. *Proc Natl Acad Sci USA* 104:3835–3840.
14. Roose T, Netti PA, Munn LL, Boucher Y, Jain RK (2003) Solid stress generated by spheroid growth estimated using a linear poroelasticity model small star, filled. *Microvasc Res* 66:204–212.
15. Araujo R, McElwain D (2004) A linear-elastic model of anisotropic tumour growth. *Eur J Appl Math* 15:365–384.
16. Bittig T, Wartlick O, Kicheva A, González-Gaitán M, Jülicher F (2008) Dynamics of anisotropic tissue growth. *New J Phys* 10:063001.
17. Goriely A, Robertson-Tessi M, Tabor M, Vandiver R (2008) Elastic growth models. *Mathematical Modeling of Biosystems*Applied Optimization, eds R Mondaini and PM Pardalos (Springer, New York), 102, pp 1–45.
18. Vandiver R, Goriely A (2009) Morpho-elastodynamics: The long-time dynamics of elastic growth. *J Biol Dyn* 3:180–195.
19. Dervaux J, Ben Amar M (2008) Morphogenesis of growing soft tissues. *Phys Rev Lett* 101:068101.
20. Ambrosi D, Preziosi L (2009) Cell adhesion mechanisms and stress relaxation in the mechanics of tumours. *Biomechan Model Mechanobiol* 8:397–413.
21. Foty R, Forgacs G, Pfleger C, Steinberg M (1994) Liquid properties of embryonic tissues: Measurement of interfacial tensions. *Phys Rev Lett* 72:2298–2301.
22. Beysens DA, Forgacs G, Glazier JA (2000) Cell sorting is analogous to phase ordering in fluids. *Proc Natl Acad Sci USA* 97:9467–9471.
23. Forgacs G, Foty RA, Shafrir Y, Steinberg MS (1998) Viscoelastic properties of living embryonic tissues: A quantitative study. *Biophys J* 74:2227–2234.
24. Nelson D, Toner J (1981) Bond-orientational order, dislocation loops, and melting of solids and smectic-a liquid crystals. *Phys Rev B* 24:363–387.
25. Théry M, Jiménez-Dalmaroni A, Racine V, Bornens M, Jülicher F (2007) Experimental and theoretical study of mitotic spindle orientation. *Nature* 447:493–496.
26. Jungbauer S, Gao H, Spatz JP, Kemkemer R (2008) Two characteristic regimes in frequency-dependent dynamic reorientation of fibroblasts on cyclically stretched substrates. *Biophys J* 95:3470–3478.
27. Fink J (2009) Mechanotransduction in mitotic spindle positioning. PhD thesis (Université Paris IX, Paris).
28. Binney JJ, Dowrick NJ, Fisher AJ, Newman MEJ (1992) *The Theory of Critical Phenomena* (Clarendon, Oxford).
29. Bryant DM, Mostov KE (2008) From cells to organs: Building polarized tissue. *Nat Rev Mol Cell Biol* 9:887–901.
30. Wang Y, Nathans J (2007) Tissue/planar cell polarity in vertebrates: New insights and new questions. *Development* 134:647–658.
31. Kruse K, Joanny JF, Jülicher F, Prost J, Sekimoto K (2005) Generic theory of active polar gels: A paradigm for cytoskeletal dynamics. *Eur Phys J E* 16:5–16.
32. de Gennes PG, Prost J (1993) *The Physics of Liquid Crystals* (Clarendon, Oxford), 2nd Ed.
33. Bedeaux D, Mazur P (1974) Brownian motion and fluctuating hydrodynamics. *Physica* 76:247–258.
34. Hoogerbrugge P, Koelman J (1992) Simulating microscopic hydrodynamic phenomena with dissipative particle dynamics. *Europhys Lett* 19:155–160.
35. Groot R, Warren P (1997) Dissipative particle dynamics: Bridging the gap between atomistic and mesoscopic simulation. *J Chem Phys* 107:4423–4435.
36. Théry M, Bornens M (2006) Cell shape and cell division. *Curr Opin Cell Biol* 18:648–657.
37. Mombach J, Glazier JA (1996) Single cell motion in aggregates of embryonic cells. *Phys Rev Lett* 76:3032–3035.
38. Guevorkian K, Colbert MJ, Durth M, Dufour S, Brochard-Wyart F (2010) Aspiration of biological viscoelastic drops. *Phys Rev Lett* 104:218101.

Supporting Information

Ranft et al. 10.1073/pnas.1011086107

SI Text

Force Dipoles in an Elastic Medium. ***Total stress and force balances.*** We consider an isotropic elastic medium with discrete force dipoles $d_{\alpha\beta}$ in the presence of an external stress $\sigma^{\text{ext}}_{\alpha\beta}$. The Hookian law is expressed as

$$\sigma^{\text{ext}}_{\alpha\beta} = C_{\alpha\beta\gamma\nu} u^{\text{ext}}_{\gamma\nu}, \qquad \text{[S1]}$$

where $u^{\text{ext}}_{\alpha\beta}$ is the strain due to external stresses. The tensor of elastic constants is given by $C_{\alpha\beta\gamma\nu} = \chi\delta_{\alpha\beta}\delta_{\gamma\nu} + 2\mu(\delta_{\alpha\gamma}\delta_{\beta\nu} - \delta_{\alpha\beta}\delta_{\gamma\nu}/3)$, where χ and μ are the bulk and shear elastic moduli, respectively. The presence of force dipoles introduces additional strain; the total strain can be written as

$$u_{\alpha\beta} = u^{\text{ext}}_{\alpha\beta} + \sum_n H_{\alpha\beta\gamma\delta}(\mathbf{r} - \mathbf{r}_n) d^{(n)}_{\gamma\delta}. \qquad \text{[S2]}$$

Here, $H_{\alpha\beta\gamma\delta}$ is the Green's function corresponding to a point dipole; see *Green's function of force dipoles in elastic media*. For an infinite elastic medium, it is given by

$$\begin{aligned} A^{-1} H_{\alpha\beta\gamma\delta}(\mathbf{r}) = &-\frac{1}{r^3}[\delta_{\alpha\beta}\delta_{\gamma\delta} - (1-2\nu)(\delta_{\alpha\gamma}\delta_{\beta\delta} + \delta_{\alpha\delta}\delta_{\beta\gamma})] \\ &+ \frac{3}{r^5}[r_\alpha r_\beta \delta_{\gamma\delta} + r_\alpha r_\gamma \delta_{\beta\delta} + r_\beta r_\gamma \delta_{\alpha\delta} + r_\gamma r_\delta \delta_{\alpha\beta} \\ &- (1-2\nu)(r_\alpha r_\delta \delta_{\beta\gamma} + r_\beta r_\delta \delta_{\alpha\gamma})] - \frac{15}{r^7} r_\alpha r_\beta r_\gamma r_\delta, \end{aligned} \qquad \text{[S3]}$$

with $A = (3\chi - \mu)/(24\pi\chi\mu)$ and Poisson's ratio $\nu = (3\chi - 2\mu)/(6\chi - 2\mu)$. The stress associated with this total strain field is

$$\sigma^{\text{el}}_{\alpha\beta} = C_{\alpha\beta\gamma\nu} u_{\gamma\nu}. \qquad \text{[S4]}$$

From the definition of the total strain $u_{\alpha\beta}$, it follows that

$$\partial_\beta \sigma^{\text{el}}_{\alpha\beta} = \sum_n \partial_\beta [d^{(n)}_{\alpha\beta} \delta(\mathbf{r} - \mathbf{r}_n)] - f^{\text{ext}}_\alpha, \qquad \text{[S5]}$$

where $f^{\text{ext}}_\alpha = -\partial_\beta \sigma^{\text{ext}}_{\alpha\beta}$ is the external force. The overall force balance for the total stress $\sigma_{\alpha\beta}$ reads

$$\partial_\beta \sigma_{\alpha\beta} + f^{\text{ext}}_\alpha = 0. \qquad \text{[S6]}$$

Therefore, we can write

$$\sigma_{\alpha\beta} = \sigma^{\text{el}}_{\alpha\beta} + \sigma^{s}_{\alpha\beta}, \qquad \text{[S7]}$$

where the contribution to the total stress from the force dipoles is given by $\sigma^{s}_{\alpha\beta} = -Q_{\alpha\beta}$. Here, we have introduced the dipole density

$$Q_{\alpha\beta}(\mathbf{r}) = \sum_n d^{(n)}_{\alpha\beta} \delta(\mathbf{r} - \mathbf{r}_n). \qquad \text{[S8]}$$

Thus, the total stress can be written as

$$\sigma_{\alpha\beta} = C_{\alpha\beta\gamma\nu} u_{\gamma\nu} - Q_{\alpha\beta}. \qquad \text{[S9]}$$

The generation of force dipoles in the system therefore implies the generation of an additional stress $\sigma^{s}_{\alpha\beta} = -Q_{\alpha\beta}$, which we refer to as source stress. In a tissue, cell division and apoptosis correspond to the addition and removal of force dipoles and lead to dynamic changes of this source stress $\sigma^{s}_{\alpha\beta}$.

Green's function of force dipoles in elastic media. Let $G_{\alpha\beta}$ denote the Green's function of an elastic medium such that the deformation field $\mathbf{u}$ due to a point force $\mathbf{f}$ at $\tilde{\mathbf{r}}$ is given by

$$u_a(\mathbf{r}) = G_{\alpha\beta}(\mathbf{r} - \tilde{\mathbf{r}}) f_\beta. \qquad \text{[S10]}$$

For any force dipole $d_{\alpha\beta}\delta(\tilde{\mathbf{r}})$ we can introduce the force distribution $f^{d}_{\alpha}(\tilde{\mathbf{r}}) = -d_{\alpha\beta}\partial_\beta \delta(\tilde{\mathbf{r}})$, which implies

$$\begin{aligned} u_a(\mathbf{r}) &= -\int \mathrm{d}^3\tilde{r} G_{\alpha\beta}(\mathbf{r} - \tilde{\mathbf{r}}) d_{\beta\gamma} \partial_{\tilde{\gamma}} \delta(\tilde{\mathbf{r}}) \\ &= +\int \mathrm{d}^3\tilde{r} \partial_{\tilde{\gamma}} G_{\alpha\beta}(\mathbf{r} - \tilde{\mathbf{r}}) d_{\beta\gamma} \delta(\tilde{\mathbf{r}}) \\ &= -\int \mathrm{d}^3\tilde{r} G_{\alpha\beta,\gamma}(\mathbf{r} - \tilde{\mathbf{r}}) d_{\beta\gamma} \delta(\tilde{\mathbf{r}}), \end{aligned} \qquad \text{[S11]}$$

where $\partial_{\tilde{\gamma}} = \partial/\partial\tilde{x}_\gamma$ and $G_{\alpha\beta,\gamma} = (\partial/\partial x_\gamma) G_{\alpha\beta}$. Here, we have used the translational invariance of an infinite medium, which is expressed by the fact that $G_{\alpha\beta}(\mathbf{r},\tilde{\mathbf{r}}) = G_{\alpha\beta}(\mathbf{r} - \tilde{\mathbf{r}})$. Thus we have

$$u_{\alpha\beta} = \frac{1}{2}(\partial_\alpha u_\beta + \partial_\beta u_\alpha) \qquad \text{[S12]}$$

$$= -\frac{1}{2}\int \mathrm{d}^3\tilde{r}\{G_{\alpha\gamma,\delta\beta}(\mathbf{r} - \tilde{\mathbf{r}}) + G_{\beta\gamma,\delta\alpha}(\mathbf{r} - \tilde{\mathbf{r}})\} d_{\gamma\delta}\delta(\tilde{\mathbf{r}}) \qquad \text{[S13]}$$

$$\equiv \int \mathrm{d}^3\tilde{r} H_{\alpha\beta\gamma\delta}(\mathbf{r} - \tilde{\mathbf{r}}) d_{\gamma\delta}\delta(\tilde{\mathbf{r}}), \qquad \text{[S14]}$$

where we have defined the force dipole Green's function $H_{\alpha\beta\gamma\delta}$. It can be obtained from the point force Green's function $G_{\alpha\beta}$ (e.g., discussed in ref. 1) as done above for the infinite isotropic elastic medium; see Eq. **S3**. (See also ref. 2 for a discussion of single cells acting as force dipoles in elastic media and corresponding Green's functions.)

Analogy with electrostatics in dielectric media. There is a formal analogy between stresses in an elastic medium in the presence of force dipoles,

$$\sigma_{\alpha\beta} = C_{\alpha\beta\gamma\nu} u_{\gamma\nu} - Q_{\alpha\beta}, \qquad \text{[S15]}$$

and electrostatics in polarizable media,

$$D_\alpha = \epsilon_0 E_\alpha + P_\alpha. \qquad \text{[S16]}$$

Here, D_α is the dielectric displacement, ϵ_0 the vacuum permittivity, E_α the electric field, and P_α the polarization. Note that $\partial_\alpha D_\alpha = \rho_{\text{ext}}$, where ρ_{ext} is the free charge density, and $P_\alpha = \sum_n p^{(n)}_\alpha \delta(\mathbf{r} - \mathbf{r}_n)$ is a dipole density; also, $\partial_\alpha E_\alpha = [\rho_{\text{ext}} - \sum_n \partial_\alpha p^{(n)}_\alpha \delta(\mathbf{r} - \mathbf{r}_n)]/\epsilon_0$. The analogy is then $\sigma_{\alpha\beta} \leftrightarrow D_\alpha$, $u_{\alpha\beta} \leftrightarrow E_\alpha$, $Q_{\alpha\beta} \leftrightarrow -P_\alpha$, $f^{\text{ext}}_\alpha \leftrightarrow -\rho^{\text{ext}}$, and $\epsilon_0 \leftrightarrow C_{\alpha\beta\gamma\nu}$. A similar analogy between the elastic deformation around dislocation lines and the magnetic field around lines of constant current has been pointed out by refs. 1 and 3.

Dynamic Force Dipole Densities. The stress and strain entering Eq. **S9** depend on a reference state with respect to which strain is defined. Even though such a reference state can no longer uniquely be defined in a tissue that is remodeled by cell division and apoptosis, differences of stress and strain still have a meaning. In general, a local flow field $\mathbf{v}(\mathbf{r},t)$ exists such that

$$u_{\alpha\beta}(t) - u_{\alpha\beta}(t_0) = \int_{t_0}^{t} \mathrm{d}t' v_{\alpha\beta}(t') \qquad \textbf{[S17]}$$

to linear order. Here, $v_{\alpha\beta} = \frac{1}{2}(\partial_\alpha v_\beta + \partial_\beta v_\alpha)$ denotes the velocity gradient of the flow. The changes in stress at time t, starting from an initial state at t_0, are given to linear order by

$$\sigma_{\alpha\beta}(t) - \sigma_{\alpha\beta}(t_0) = C_{\alpha\beta\gamma\nu}[u_{\gamma\nu}(t) - u_{\gamma\nu}(t_0)] - Q_{\alpha\beta}(t) + Q_{\alpha\beta}(t_0). \qquad \textbf{[S18]}$$

Alternatively, we can express the dynamic change of the stress as a differential equation:

$$\frac{\mathrm{D}}{\mathrm{D}t}\sigma_{\alpha\beta} = C_{\alpha\beta\gamma\nu}v_{\gamma\nu} + \frac{\mathrm{D}}{\mathrm{D}t}\sigma^{s}_{\alpha\beta}. \qquad \textbf{[S19]}$$

Here, $(\mathrm{D}/\mathrm{D}t)\sigma_{\alpha\beta} = \partial_t\sigma_{\alpha\beta} + v_\gamma\partial_\gamma\sigma_{\alpha\beta} + \omega_{\alpha\gamma}\sigma_{\gamma\beta} + \omega_{\beta\gamma}\sigma_{\alpha\gamma}$ is the convected corotational time derivative that captures geometric nonlinearities and $\omega_{\alpha\beta} = (1/2)(\partial_\alpha v_\beta - \partial_\beta v_\alpha)$ is the vorticity of the flow. Eqs. **S18** and **S19** are independent of any reference state, in contrast to Eq. **S9**.

Nonlinearities. Eq. **S19** describing the changes of stress includes geometric nonlinearities in the convected corotational time derivatives. In order to develop a coherent description of nonlinearities, we also have to add physical nonlinearities of the same order. Therefore, the stress changes behave up to second order as

$$\frac{\mathrm{D}}{\mathrm{D}t}\sigma_{\alpha\beta} = C_{\alpha\beta\gamma\nu}v_{\gamma\nu} + D_1\tilde{v}_{\alpha\gamma}\tilde{v}_{\gamma\beta} + D_2 v_{\gamma\gamma}v_{\delta\delta}\delta_{\alpha\beta} + D_3\tilde{v}_{\alpha\beta}v_{\gamma\gamma} + \frac{\mathrm{D}}{\mathrm{D}t}\sigma^{s}_{\alpha\beta}. \qquad \textbf{[S20]}$$

Here, the phenomenological coefficients D_i describe the magnitude of nonlinear terms. The source stress also has to be expanded to second order in the stress:

$$\frac{\mathrm{D}}{\mathrm{D}t}\sigma^{s}_{\alpha\beta} = -\frac{\tilde{\sigma}_{\alpha\beta}}{\tau_a} - \frac{\sigma_{\gamma\gamma}}{\tau}\delta_{\alpha\beta} - E_1\tilde{\sigma}_{\alpha\gamma}\tilde{\sigma}_{\gamma\beta} - E_2\sigma_{\gamma\gamma}\sigma_{\delta\delta}\delta_{\alpha\beta} - E_3\tilde{\sigma}_{\alpha\beta}\sigma_{\gamma\gamma}, \qquad \textbf{[S21]}$$

with coefficients E_i of the nonlinear terms.

Numerical Simulations. Here, we provide details of the simulation scheme for dynamic tissues and our parameter choices.

Dissipative particle dynamics for growing tissues. We describe the tissue by an ensemble of interacting cells, where each cell is represented by two positional variables. This representation allows us to capture the anisotropy of cell growth and division. In the following, we refer to these positional variables as point particles. The two particles that define a cell interact with the particles of surrounding cells via a pair potential that accounts for cell mechanics and cell–cell adhesion. The potential depends on the distance r between the interacting particles and contains a short-range repulsive and a midrange attractive contribution. Particles farther apart than a certain cutoff length R_{pp} do not interact. The potential is given by

$$V^{CC}(r) = \begin{cases} \frac{f_0 R_{\mathrm{pp}}^5}{4r^4} + (f_0 + f_1)\cdot r + V_0 & r \le R_{\mathrm{pp}}, \\ 0 & r > R_{\mathrm{pp}}. \end{cases} \qquad \textbf{[S22]}$$

Here, f_0 and f_1 are coefficients that describe repulsion and attraction, respectively, and $V_0 = -(5f_0/4 + f_1)\cdot R_{\mathrm{pp}}$ is chosen such that the potential vanishes continuously at $r = R_{\mathrm{pp}}$. In addition, two particles that belong to the same cell interact via a potential that describes axial cell growth. The growth potential is given by

$$V^{G}(r) = \frac{B}{r + r_0}, \qquad \textbf{[S23]}$$

where B is an expansion strength and r_0 a characteristic length. Note that particles that belong to the same cell do not interact via the cell–cell potential V^{CC}.

Cell division and apoptosis are implemented as follows. When the distance between the two particles of a cell exceeds a size threshold R_{c}, the cell divides. After the division, each of the original particles constitutes a daughter cell. For each daughter cell, a new particle is then placed randomly within a short distance r_{c} from the original particle. Whereas cell division is implemented in this deterministic manner, apoptosis is included by removing cells randomly at a constant rate k_{a}.

Cell–cell friction is described by using dissipative particle dynamics (DPD) (4–6). We use an implementation of DPD where each particle is assigned a mass m and is subject to friction forces due to motion relative to particles of neighboring cells (friction γ_{CC}) and relative to the second particle of the same cell (friction γ_G). In addition, we introduce a friction γ_B with respect to a background medium in order to dampen movement of the center of mass of the whole system induced by cell division or apoptosis.

Furthermore, random forces are introduced to account for noise. For simplicity, we choose white noise obeying a fluctuation–dissipation relation with respect to the friction coefficients γ_{CC}, γ_G, and γ_B, so that the noise strength can be characterized by an effective temperature T_{noise}.

Standard tissue and units. The parameters of our model are specified with respect to a unit of length l_0, a unit of time t_0, and a unit of pressure p_0. We define a standard tissue that we refer to when exploring the parameter space. The parameter values of the standard tissue in the units l_0, t_0, and p_0 are given in Table S1. These values are chosen such that important properties of the standard tissue take approximately the value of 1 in the units l_0, t_0, and p_0: For the standard tissue confined in a box of volume V_{box} with periodic boundary conditions, we obtain the cell density $\rho = \frac{\langle N\rangle}{V_{\mathrm{box}}} \simeq l_0^{-3}$, where $\langle N\rangle$ denotes the average number of cells at the homeostatic state. Moreover, for the average cell division rate k_d of the standard tissue in the homeostatic state, we have $k_d \simeq t_0^{-1}$. Finally, the homeostatic pressure p_h of the standard tissue is $p_h \simeq p_0$. Parameter values of simulations that deviate from the standard case are specified relative to the standard values denoted by an asterisk; i.e., $B^* = 0.5$ denotes an expansion strength which is decreased by a factor of 2 with respect to the standard tissue.

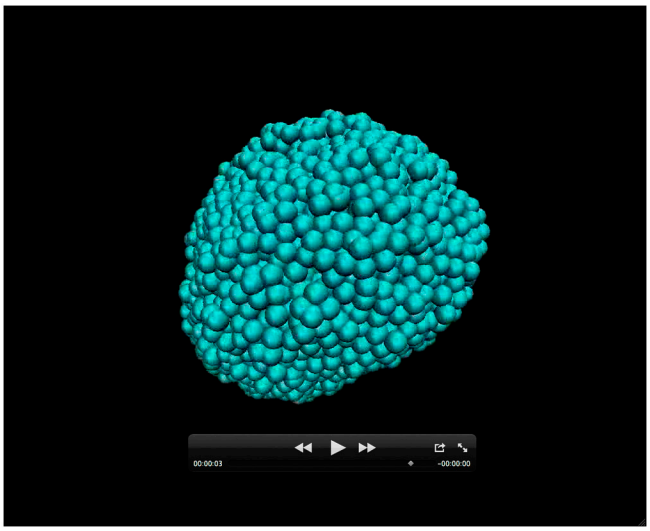
Boundary conditions and measurement procedures. To measure the diffusion constant of cells in our simulations, the tissue is grown to its homeostatic state in a cubic compartment with lateral dimension $4R_{\mathrm{pp}}$ by using periodic boundary conditions in all three directions. We then track particles and determine their mean squared displacement (MSD), which shows a linear behavior in time. The diffusion constant is obtained from a linear fit to the slope of the MSD.

For a measurement of the homeostatic pressure and the tissue viscosity, the tissue is grown between two walls with fixed distance and periodic boundary conditions in the plane. We chose a bounce-back boundary condition at the walls in order to mimic a no-slip boundary condition (7). In short, the velocity of a particle is reversed the very moment it hits the wall. In the homeo-

static state, the homeostatic pressure is determined from the momentum exchange between the tissue and the walls.

In order to determine the tissue viscosity, the top wall moves with a prescribed velocity relative to the bottom wall. In this case, the bounce-back boundary condition at the top wall is implemented in its rest frame. The wall motion creates a shear stress σ_{xz} in the tissue. Both the shear stress and the pressure are accessible by measuring the momentum exchange of particles with the wall. (In the shear simulations, we choose a box size of $5R_{\mathrm{pp}}$ and $\gamma_B = 0$.) However, because bounce-back boundary conditions do not provide perfect no-slip boundary conditions, we measure and fit the velocity profile $v_x(z)$ in the tissue directly. Then the shear viscosity is given by $\eta = \sigma_{xz}/(\partial_z v_x)$.

1. Landau LD, Lifshitz EM (1986) *Theory of Elasticity*, Course of Theoretical Physics, Vol 7 (Butterworth–Heinemann, Oxford), 3rd Ed.
2. Bischofs IB, Safran SA, Schwarz US (2004) Elastic interactions of active cells with soft materials. *Phys Rev E* 69:021911.
3. Pershan PS, Prost J (1975) Dislocation and impurity effects in smectic-a liquid crystals. *J Appl Phys* 46:2343–2353.
4. Hoogerbrugge P, Koelman J (1992) Simulating microscopic hydrodynamic phenomena with dissipative particle dynamics. *Europhys Lett* 19:155–160.
5. Groot R, Warren P (1997) Dissipative particle dynamics: Bridging the gap between atomistic and mesoscopic simulation. *J Chem Phys* 107:4423–4435.
6. Nikunen P, Karttunen M, Vattulainen I (2003) How would you integrate the equations of motion in dissipative particle dynamics simulations? *Comput Phys Commun* 153:407–423.
7. Allen MP, Tildesley DJ (1989) *Computer Simulation of Liquids* (Clarendon, Oxford).



Movie S1. An example of a tissue growth simulation by dissipative particle dynamics. The simulation starts from one cell and proceeds to 10,000 cells by using standard parameters.
Movie S1 (MOV)

Table S1. Standard tissue parameters

Parameter	Value	Unit	Description
f_0	$1.398 \cdot 10^{-1}$	$p_0 l_0^2$	Repulsive cell–cell potential coefficient
f_1	$2.918 \cdot 10^{-1}$	$p_0 l_0^2$	Attractive cell–cell potential coefficient
R_{pp}	1.387	l_0	Range of pair potentials
B	5.616	$p_0 l_0^4$	Cellular expansion pressure constant I
r_0	1.387	l_0	Cellular expansion pressure constant II
R_c	1.11	l_0	Threshold distance for cell division
r_c	$1.387 \cdot 10^{-5}$	l_0	Distance at which new particles are placed
k_a	1	t_0^{-1}	Rate of cell death
γ_{CC}	$4.206 \cdot 10^{-2}$	$p_0 l_0 t_0$	Intracellular dissipation constant between particles of the same cell
γ_G	$2.103 \cdot 10^{-2}$	$p_0 l_0 t_0$	Intercellular dissipation constant between particles of different cells but of the same tissue
γ_B	$4.206 \cdot 10^{-5}$	$p_0 l_0 t_0$	Background friction coefficient
R_t	1.387	l_0	Range of dissipative friction forces when the cell divides
$k_{\mathrm{B}} T_{\mathrm{noise}}$	$8.096 \cdot 10^{-3}$	$p_0 l_0^3$	Noise strength in the tissue
m	$4.206 \cdot 10^{-6}$	$p_0 l_0 t_0^2$	Mass of particle

This table lists all parameters of our simulation and gives their values in simulation units l_0, t_0, and p_0 for the standard tissue.