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Supplementary Information for

Hydraulic and electric control of cell spheroids

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Supporting Information Text

Tissues as active two-component fluids

Mass conservation in growing tissues. In this work, we describe the tissue as a two-component system with (i) a cell phase that accounts for cells and the surrounding extra-cellular matrix, and (ii) the interstitial fluid that permeates the cell phase. Such a description has been introduced in Ref. (1). Here, we briefly review this approach to clarify the conceptual basis of Eq. [6] in the main text.

The cell phase is characterized by a cell mass $m^{\rm c}$, a cell number density $n^{\rm c}$, and a cell volume $\Omega^{\rm c}$, and we introduce similarly $m^{\rm f}$, $n^{\rm f}$ and $\Omega^{\rm f}$ for the interstitial fluid. The assumption that the cells and the fluid fill completely the space is written as:

$$n^{\rm c}\Omega^{\rm c} + n^{\rm f}\Omega^{\rm f} = 1\,. \qquad [1]$$

We use this assumption to define the cell volume fraction $\phi = n^{\rm c}\Omega^{\rm c}$ and the fluid volume fraction $n^{\rm f}\Omega^{\rm f} = 1-\phi$. Mass conservation in the tissue reads:

$$\partial_t \rho + \partial_\alpha j_\alpha = 0\,, \qquad [2]$$

where $\rho = m^{\rm c}n^{\rm c} + m^{\rm f}n^{\rm f}$ is the tissue density and $j_\alpha = m^{\rm c}n^{\rm c}v^{\rm c}_\alpha + m^{\rm f}n^{\rm f}v^{\rm f}_\alpha$ is the total mass flux with $v^{\rm c,f}_\alpha$ the cell and interstitial fluid velocities. As a consequence of cell division and death, cell number is not conserved but obeys a continuity equation:

$$\partial_t n^{\rm c} + \partial_\alpha (n^{\rm c} v^{\rm c}_\alpha) = n^{\rm c}(k_{\rm d} - k_{\rm a})\,, \qquad [3]$$

where $k_{\rm d}$ and $k_{\rm a}$ are the rates of cell division and apoptosis respectively. Using mass conservation [2], the cell continuity equation [3], and assuming a constant fluid particle mass, we obtain the continuity equation for the fluid particle density (2):

$$\partial_t n^{\rm f} + \partial_\alpha (n^{\rm f} v^{\rm f}_\alpha) = -\frac{m^{\rm c}}{m^{\rm f}} n^{\rm c}(k_{\rm d} - k_{\rm a}) - \frac{n^{\rm c}}{m^{\rm f}}\frac{{\rm d}}{{\rm d}t} m^{\rm c}, \qquad [4]$$

where ${\rm d}/{\rm d}t = \partial_t + v^{\rm c}_\gamma \partial_\gamma$ is the convected time derivative with respect to the cell flow. The above equation implies that a cell of mass $m^{\rm c}$ can be converted into $m^{\rm c}/m^{\rm f}$ fluid particles and vice versa when cells die or divide.

We define the total volume flux $v_\alpha = n^{\rm c}\Omega^{\rm c}v^{\rm c}_\alpha + n^{\rm f}\Omega^{\rm f}v^{\rm f}_\alpha$. Using Eqs. [1], [3] and [4] we obtain the following expression for its divergence (1):

$$\begin{aligned}\partial_\alpha v_\alpha = &\left(1 - \frac{\rho^{\rm c}}{\rho^{\rm f}}\right)\phi\left(k_{\rm d} - k_{\rm a} + \frac{1}{\Omega^{\rm c}}\frac{{\rm d}}{{\rm d}t}\Omega^{\rm c}\right)\\ &- \frac{\phi}{\rho^{\rm f}}\frac{{\rm d}}{{\rm d}t}\rho^{\rm c} - \frac{1-\phi}{\rho^{\rm f}}(\partial_t + v^{\rm f}_\alpha\partial_\alpha)\rho^{\rm f}\,,\end{aligned} \qquad [5]$$

where we have defined $\rho^{\rm c} = m^{\rm c}/\Omega^{\rm c}$ and $\rho^{\rm f} = m^{\rm f}/\Omega^{\rm f}$ the cell and fluid particle mass densities. Assuming that cell and fluid densities are constant and that $\rho^{\rm c} = \rho^{\rm f}$, the previous equation simplifies to yield $\partial_\alpha v_\alpha = 0$ (1). In the presence of the drain, which imposes a nonvanishing fluid velocity at the inner boundary of the spheroid, the integration of the total flow incompressibility in spherical coordinates yields Eq. [6] in the main text.

Constitutive equations for the isotropic and anisotropic parts of the cell stress. Following Refs. (1–3), we derive the constitutive equations for the cell stress $\sigma^{\rm c}_{\alpha\beta}$ for a permeated tissue in the presence of electric fields. We decompose the cell stress tensor into an isotropic contribution $\sigma^{\rm c}$ and a traceless part $\tilde\sigma^{\rm c}_{\alpha\beta}$, such that $\sigma^{\rm c}_{\alpha\beta} = \tilde\sigma^{\rm c}_{\alpha\beta} + \sigma^{\rm c}\delta_{\alpha\beta}$.

We first discuss the isotropic cell stress. Cell volume $\Omega^{\rm c}$ and cell volume fraction ϕ are in general functions of the isotropic cell stress $\sigma^{\rm c}$, the anisotropic cell stress $\tilde\sigma^{\rm c}_{\alpha\beta}$, the electric field E_α, and the velocity difference $V_\alpha = v^{\rm c}_\alpha - v^{\rm f}_\alpha$. A general equation of state for the cell volume can therefore be written as:

$$\Omega^{\rm c} = \Omega^{\rm c}(\sigma^{\rm c}, q_{\alpha\beta}\tilde\sigma^{\rm c}_{\alpha\beta}, p_\alpha E_\alpha, p_\alpha V_\alpha)\,, \qquad [6]$$

and a similar expression for the cell volume fraction ϕ. Since $n^{\rm c} = \phi/\Omega^{\rm c}$, we can use the equation of state [6] to write the time dependence of the cell number density:

$$\frac{1}{n^{\rm c}}\frac{{\rm d}n^{\rm c}}{{\rm d}t} = -\frac{1}{\chi}\frac{{\rm d}\sigma^{\rm c}}{{\rm d}t} - \frac{1}{\chi_0}\frac{{\rm d}(q_{\alpha\beta}\tilde\sigma^{\rm c}_{\alpha\beta})}{{\rm d}t} - \frac{1}{\chi_1}\frac{{\rm d}(p_\alpha E_\alpha)}{{\rm d}t} - \frac{1}{\chi_2}\frac{{\rm d}(p_\alpha V_\alpha)}{{\rm d}t}\,, \qquad [7]$$

where $\chi = n^{\rm c}(\partial n^{\rm c}/\partial\sigma^{\rm c})^{-1}$ and $\chi_0 = n^{\rm c}[\partial n^{\rm c}/\partial(q_{\alpha\beta}\tilde\sigma^{\rm c}_{\alpha\beta})]^{-1}$, denotes the isotropic and anisotropic compressibilities of the cells, and where we have defined the other compressibility coefficients as $\chi_1 = n^{\rm c}[\partial n^{\rm c}/\partial(p_\alpha E_\alpha)]^{-1}$, and $\chi_2 = n^{\rm c}[\partial n^{\rm c}/\partial(p_\alpha V_\alpha)]^{-1}$. To write the constitutive equation for the isotropic stress in a closed form, we now eliminate $n^{\rm c}$. For this purpose the cell continuity equation [3] can be rewritten as:

$$\frac{1}{n^{\rm c}}\frac{{\rm d}n^{\rm c}}{{\rm d}t} = -v^{\rm c}_{\gamma\gamma} + k_{\rm d} - k_{\rm a}\,, \qquad [8]$$

and we specify a constitutive equation for the net growth rate of the tissue $k_\mathrm{d} - k_\mathrm{a}$. This growth rate in general depends on the isotropic stress σ^c and may also depend on $q_{\alpha\beta}\tilde{\sigma}^\mathrm{c}_{\alpha\beta}$, $p_\alpha E_\alpha$ and $p_\alpha V_\alpha$. In the absence of anisotropic stresses, electric fields and flows, a constant cell density is achieved when cell death compensates cell division. The resulting isotropic cell pressure is the homeostatic pressure P^c_h. To linear order, the net growth rate near the homeostatic pressure reads (1, 3):

$$k_\mathrm{d} - k_\mathrm{a} = \bar{\eta}^{-1}(P^\mathrm{c}_\mathrm{h} + \sigma^\mathrm{c} + \nu_0\tilde{\sigma}^\mathrm{c}_{\alpha\beta}q_{\alpha\beta} + \nu_1 p_\alpha E_\alpha + \nu_2 p_\alpha V_\alpha)\,, \tag{9}$$

where $\bar{\eta}$ is a constant and will be identified as the bulk viscosity in the following, ν_0 is a dimensionless coefficient that takes into account the possible dependence of the growth rate on the anisotropic part of the stress, ν_1 characterizes the influence of the electric field on the growth rate and ν_2 is a coefficient accounting for the effects of the relative motion of the cells and the interstitial fluid to the growth rate. Using Eqs. [7]-[9], we obtain a general constitutive equation for the isotropic cell stress:

$$\begin{aligned}&\left(1+\tau\frac{\mathrm{d}}{\mathrm{d}t}\right)(\sigma^\mathrm{c}+P^\mathrm{c}_\mathrm{h}) + \nu_0\left(1+\tau'\frac{\mathrm{d}}{\mathrm{d}t}\right)\tilde{\sigma}^\mathrm{c}_{\alpha\beta}q_{\alpha\beta}\\ &+\nu_1\left(1+\tau_1\frac{\mathrm{d}}{\mathrm{d}t}\right)p_\alpha E_\alpha + \nu_2\left(1+\tau_2\frac{\mathrm{d}}{\mathrm{d}t}\right)p_\alpha V_\alpha = \bar{\eta}v^\mathrm{c}_{\gamma\gamma}\,,\end{aligned} \tag{10}$$

where $\tau = \bar{\eta}/\chi$ is and $\tau' = \bar{\eta}/\chi_0$ are the isotropic and anisotropic relaxation rates, $\tau_1 = \bar{\eta}/\chi_1$ the relaxation rate associated with the electric field, $\tau_2 = \bar{\eta}/\chi_2$ the relaxation rate arising from a velocity difference. At long times that we consider in the main text, we neglect the relaxation processes and Eq. [10] reduces to

$$\sigma^\mathrm{c} + P^\mathrm{c}_\mathrm{h} = \bar{\eta}v^\mathrm{c}_{\gamma\gamma} - \nu_0\tilde{\sigma}^\mathrm{c}_{\alpha\beta}q_{\alpha\beta} - \nu_1 p_\alpha E_\alpha - \nu_2 p_\alpha V_\alpha\,, \tag{11}$$

which is Eq. [2a] in the main text. In this long-time limit, cells are described as an active viscous fluid where $\bar{\eta}$ is revealed as an effective bulk viscosity due to cell division and death.

We now discuss the anisoptropic part of the cell stress $\tilde{\sigma}^\mathrm{c}_{\alpha\beta}$. We assume that the tissue behaves as an isotropic elastic material in the absence of cell division and apoptosis. When these events are considered, a reference state for the stress cannot be defined and we therefore express the changes of stress as a differential equation (1):

$$\frac{\mathrm{D}}{\mathrm{D}t}\tilde{\sigma}^\mathrm{c}_{\alpha\beta} = 2\mu\tilde{v}^\mathrm{c}_{\alpha\beta} + \frac{\mathrm{D}}{\mathrm{D}t}\tilde{\sigma}^{\mathrm{c,a}}_{\alpha\beta}\,, \tag{12}$$

where $(\mathrm{D}/\mathrm{D}t)\sigma^\mathrm{c}_{\alpha\beta} = \partial_t\sigma^\mathrm{c}_{\alpha\beta} + v^\mathrm{c}_\gamma\partial_\gamma\sigma^\mathrm{c}_{\alpha\beta} + \omega_{\alpha\gamma}\sigma^\mathrm{c}_{\gamma\beta} + \omega_{\beta\gamma}\sigma^\mathrm{c}_{\alpha\gamma}$ refers to the corotational time derivative with respect to the cell flow with $\omega_{\alpha\beta} = (\partial_\alpha v^\mathrm{c}_\beta - \partial_\beta v^\mathrm{c}_\alpha)/2$ the cell flow vorticity. This corotational derivative allows us to define a constitutive equation which does not depend on a frame of reference. We have also introduced the shear-rate tensor $\tilde{v}_{\alpha\beta}$, and, assuming that the tissue is isotropic in terms of its elastic properties, we have introduced the cell shear modulus μ. The stress stemming from active processes is denoted by by $\tilde{\sigma}^{\mathrm{c,a}}_{\alpha\beta}$. Note that for the shear mode, cell neighbor exchanges (T1 processes) play a role similar to cell division and death. This is why the tissue shear viscosity is much smaller than the bulk viscosity, which requires division and death. In general, this term can depend on any of the traceless symmetric tensors in our model, such that at linear order we write:

$$\frac{\mathrm{D}}{\mathrm{D}t}\tilde{\sigma}^{\mathrm{c,a}}_{\alpha\beta} = -\frac{1}{\tau_\mathrm{a}}\left(\tilde{\sigma}^\mathrm{c}_{\alpha\beta} - \zeta q_{\alpha\beta} + \nu_3[E_\alpha p_\beta]_\mathrm{st} + \nu_4[V_\alpha p_\beta]_\mathrm{st}\right)\,. \tag{13}$$

where τ_a characterizes the anisotropic stress relaxation time. The constitutive equation for the anisotropic stress finally reads (2, 4):

$$\left(1+\tau_\mathrm{a}\frac{\mathrm{D}}{\mathrm{D}t}\right)\tilde{\sigma}^\mathrm{c}_{\alpha\beta} = 2\eta\tilde{v}^\mathrm{c}_{\alpha\beta} + \zeta q_{\alpha\beta} - \nu_3[E_\alpha p_\beta]_\mathrm{st} - \nu_4[V_\alpha p_\beta]_\mathrm{st}\,, \tag{14}$$

where we have defined the effective tissue shear viscosity $\eta = \mu\tau_\mathrm{a}$. In the main text we consider the long-time limit and the previous equation becomes:

$$\tilde{\sigma}^\mathrm{c}_{\alpha\beta} = 2\eta\tilde{v}^\mathrm{c}_{\alpha\beta} + \zeta q_{\alpha\beta} - \nu_3[E_\alpha p_\beta]_\mathrm{st} - \nu_4[V_\alpha p_\beta]_\mathrm{st}\,, \tag{15}$$

which is Eq. [2b] in the main text.

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