# Physics of multicellular systems

From cell to tissue mechanics

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# Contents

# Chapter 1

# Continuous models of tissues - tissue growth

This chapter focuses on tissues studied on time-scales longer than the cell cycle (typically several days), where cell divisions and apoptosis need to be taken into account in the mechanical description. Cell division is essential for the morphogenesis of embryonic tissues and organs, which need to grow to adopt their adult size, for the permanent renewal of cells in most tissues, like in the gut, and is also one of the driving force of cancerous tumors, which grow against other tissues.

In the next, we will focus mainly on the last type of tissues, in the form of multicellular spheroids made of immortalized cancerous cell lines, which can be studied *in vitro*.

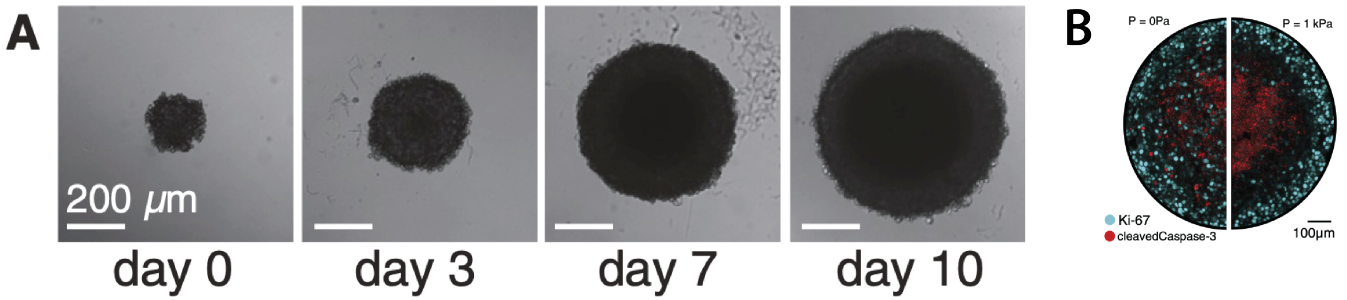


**Figure 1.1:** Growth of a cellular spheroid. (A) Snapshots of the time evolution of a spheroid made of CT26 mouse carcinoma cells [2]. (B) Distribution of proliferation and apoptosis in a spheroid (cyan corresponds to proliferation, red to apoptosis) in a tissue after 4 days of growth, with an isotropic stress of 1kPa for the right one [3].

## 1.1 Unconstrained growth of a spheroid

We start with simple considerations on a spherical spheroid.

### 1.1.1 Population dynamics

We consider a population of cells, the number of which $n$ varies by division and apoptosis, of respective rates $k_d$ and $k_a$. We can write a master equation describing the time evolution of the probability that the population has $n$ cells

$$\frac{\partial p_n}{\partial t} = k_d\big[(n-1)p_{n-1} - np_n\big] + k_a\big[(n-1)p_{n+1} - np_n\big] \tag{1.1}$$

Multiplying by $n$ and summing on all $n$, we get

$$\sum_n n\frac{\partial p_n}{\partial t} = k_d \sum_n \left\{n(n-1)p_{n-1} - n^2 p_n\right\} + k_a \sum_n \left\{n(n+1)p_{n+1} - n^2 p_n\right\}$$

One should be more cautious about the special cases $n = 0$ and $n = 1$, but we leave this reflection to the reader. We obtain thus a dynamic equation for the mean value $\langle n\rangle$

$$\begin{aligned}\frac{\partial \langle n\rangle}{\partial t} &= k_d\left[\,\langle n(n+1)\rangle - \left\langle n^2\right\rangle\,\right] + k_a\left[\,\langle n(n-1)\rangle - \left\langle n^2\right\rangle\,\right] \\ &= (k_d - k_a)\langle n\rangle \end{aligned} \tag{1.2}$$

If $k_d > k_a$, the mean number of cells grows exponentially as $\langle n\rangle = n_0 e^{(k_d-k_a)t}$

We can calculate also the variance (and more generally here all higher moments with a generating function), by multiplying the equation by $n^2$ and summing on $n$ again

$$\begin{aligned}\frac{\partial \left\langle n^2\right\rangle}{\partial t} &= k_d\Big[\,\left\langle n(n+1)^2\right\rangle - \left\langle n^3\right\rangle\,\Big] + k_a\Big[\,\left\langle n(n-1)^2\right\rangle - \left\langle n^3\right\rangle\,\Big] \\ &= 2\left\langle n^2\right\rangle(k_d - k_a) + (k_d + k_a)\langle n\rangle \end{aligned} \tag{1.3}$$

The fluctuation around the mean value is $\delta n = n - \langle n\rangle$, and the variance is obtained as

$$\begin{aligned}\frac{\partial \left\langle \delta n^2\right\rangle}{\partial t} &= \frac{\partial \left\langle n^2\right\rangle}{\partial t} - 2\langle n\rangle\frac{\partial \langle n\rangle}{\partial t} \\ &= \langle n\rangle(k_d + k_a) + 2\left\langle \delta n^2\right\rangle(k_d - k_a) \end{aligned} \tag{1.4}$$

If at a time $t$ we have exactly $n$ cells, then $\left\langle \delta n^2\right\rangle = 0$ and in the following times $\Delta t \ll 1$

$$\left\langle \delta n^2(t+\Delta t)\right\rangle = (k_d + k_a)n\Delta t,$$

such that $\delta n$ has a diffusive behavior, but where the "diffusion" coefficient depends on the value of $n$ at the time $t$ of the measure.

Such behavior can also be described by a (chemical) Langevin equation

$$\frac{dn}{dt} = (k_d - k_a)n + \sqrt{(k_d + k_a)n}\,\xi(t), \tag{1.5}$$

where $\xi(t)$ is a white noise with zero mean $\langle \xi(t)\rangle = 0$ and delta-correlated in time $\langle \xi(t)\xi(t')\rangle = \delta(t-t')$. This supposes that we work at long times compared to the typical division/apoptosis time, such that the noise is not correlated in time,nor the different divisions or apoptosis events.

If one considers a small volume $V$ around the position $\boldsymbol{r}$, we can similarly write an equation for the density $\rho(\boldsymbol{r},t) \equiv \frac{n}{V}$, including the flux of cells $\boldsymbol{r}$

$$\frac{\partial \rho}{\partial t} + \boldsymbol{\nabla}(\rho \boldsymbol{v}) = (k_d - k_a)\rho + \sqrt{\rho(k_d + k_a)}\,\xi(\boldsymbol{r},t) \tag{1.6}$$

with

$$\left\langle \xi(\boldsymbol{r},t)\xi(\boldsymbol{r}',t')\right\rangle = \delta(t-t')\delta(\boldsymbol{r}-\boldsymbol{r}').$$

### 1.1.2 Application: growth of an incompressible spheroid

We consider a spheroid made of cells considered as a single component of density $\rho = \text{cte}$ and we neglect noise. The (1.6) reduces then simply to $\boldsymbol{\nabla} \cdot \boldsymbol{v} = k_d - k_a \equiv k$, and eventhough the tissue is incompressible, the divergence of the velocity is not zero (unless $k_d = k_a$ exactly). There is therefore a constant flux of material in the spheroid.

We consider that the division properties of the spheroid depend on the radius $r$ only, and that cells divide in an external layer of thickness $\lambda$, and undergo apoptosis in the center, where the nutrient concentration is small, as depicted schematically on Fig. 1.2a.

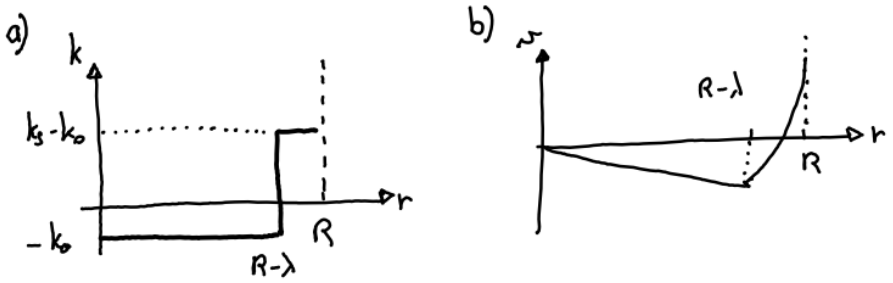


**Figure 1.2:** Growth of an incompressible spheroid. (a) Profile of the proliferation rate in the spheroid. (b) Radial velocity profile in the spheroid.

The equation can be written in spherical coordinates, which reads

$$\frac{1}{r^2}\frac{\partial}{\partial r}(r^2 v) = k(r).$$

If $k$ is constant, a solution is $v = \frac{kr}{3} + \frac{A}{r^2}$. In the central region of the spheroid, we have $v(r) = -k_0 \frac{r}{3}$ and $A = 0$ to avoid divergence in the center $r = 0$ and in the external region $v = (k_s - k_0)\frac{r}{3} - \frac{k_s (R_\lambda)^3}{3r^2}$ by continuity of the velocity. The profile of the velocity is sketched on Fig. 1.2b.

Over growth, $v(R) > 0$ is positive and decreases in time to eventually vanish in stationary state. At the surface, we have

$$v(R) = \frac{dR}{dt} = (k_s - k_0)\frac{R}{3} - k_s \frac{(R-\lambda)^3}{3R^2} \tag{1.7}$$

The number of cells is $\rho \frac{4}{3}\pi R^3 = N$. Multiplying the equation above by $4\pi R^2 \rho$, we get

$$\frac{dN}{dt} = -k_0 N + k_s N_s, \qquad \text{with} \quad N_s = \rho(V - V_\lambda) \tag{1.8}$$

where $V_\lambda = \frac{4}{3}\pi R^3 - \frac{4}{3}\pi (R-\lambda)^3$ is the volume of the external layer of thickness $\lambda$.

- if $R < \lambda$, $\frac{dR}{dt} = (k_s - k_0)\frac{R}{3}$: we get an exponential growth of the tissue (contributed only by the external layer).
- if $R \gg \lambda$, $\frac{dR}{dt} = k_s \lambda - \frac{k_0 R}{3}$, giving $R = R_0 \left[1 - e^{-k_0 t/3}\right]$, such that growth is linear at the beginning ($t \ll k_0^{-1}$) and saturates at $R_0 = \frac{3\lambda k_s}{k_0}$.

Experimentally, the results above have been observed in growing spheroids made of mouse colon carcinoma cell lines (CT26) [3, 4], as shown on Figs. 1.3 and 1.4.

## 1.2 Coupling between stress and proliferation

### 1.2.1 Homeostatic pressure

In general, one may expect a coupling between the rate of tissue growth and the local density: at higher density, the tissue may reduce its proliferation rate. In our continuous settings, this can be expressed by a

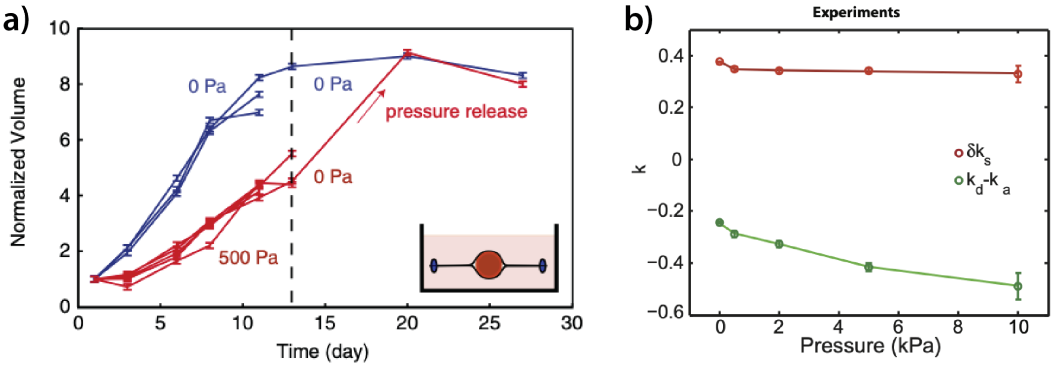


**Figure 1.3:** Growth of a cellular spheroid under stress [3]. (A) Time evolution of the volume of a spheroid with no stress and under stress. (B) Dependence of the rate of proliferation at the surface and in the bulk of a spheroid as function of the isotropic stress applied.

simple linear relationship

$$k_d - k_a = -\kappa \frac{\rho - \rho_H}{\rho_H} \tag{1.9}$$

where $\kappa$ is an inverse timescale which quantifies the sensitivity of the tissue proliferation to local density and $\rho_H$ is the density for which the division and apoptosis rates exactly balance each other, see Fig. 1.4a. The equation above can be seen as the first term in a Taylor expansion of the rate of cell division and apoptosis in the cell density.

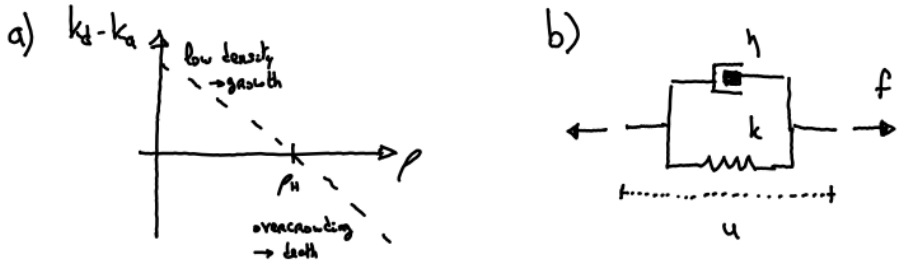


**Figure 1.4:** (a) Proliferation rate $k_d - k_a$ in the tissue as function of the local density $\rho$. (b) Rheological diagram of a Maxwell viscoelastic material of viscosity $\eta$ and elasticity $k$.

In parallel, one may also expect the isotropic part of the stress to be sensitive to cell density. This can be captured by an equation of state for the pressure $P$ in the tissue:

$$P = P_H + \chi \frac{\rho - \rho_H}{\rho_H} \tag{1.10}$$

where $\chi$ is a bulk elastic modulus. This equation simply states that cells have a preferred cell volume (or area in 2D), and deviations from this volume generates a pressure. The pressure $P_H$ is called homeostatic pressure, and is the pressure obtained at the homeostatic point $\rho = \rho_H$, where cell death and cell division exactly balance each other.

While the (1.10) corresponds to an elastic behavior, the effect of cell divisions and cell death on long timescales is to fluidify the tissue, as nicely predicted in [5].

Combining the two equations (1.9) and (1.10) with the cell density balance (1.6) (neglecting the noise term):

$$\frac{DP}{Dt} + \kappa \frac{\rho}{\rho_H}\left(P - P_H\right) = -\chi \frac{\rho}{\rho_H} \partial_k v_k \tag{1.11}$$

where $\frac{DP}{Dt} = \partial_t P + v_k \partial_k P$ is the convected (or Lagrangian) time derivative of the pressure. In fact this equation is analog, for $\rho \sim \rho_H$ to the one describing a Maxwell viscoelastic material $\left(1 + \tau \frac{d}{dt}\right) = \eta \frac{du}{dt}$, of

viscoelastic timescale $\tau = \eta/k$, as depicted on Fig. 1.4b. Here the equivalent of the viscoelastic timescale, above which the tissue transitions from a solid to a fluid behavior, is $1/\kappa$ and its effective viscosity $\chi/\kappa$.

### 1.2.2 Application: expansion of a proliferating confined tissue

As an application, we study here the expansion of a 2-dimensional profilerating tissue along the x-direction, confined in the y-direction between two plates, as depicted on the Fig. 1.5. It is supposed to have an infinite extension in the direction $x \to +\infty$, has a velocity $v_x$ along $x$ and a moving boundary of constant velocity $v_0$.

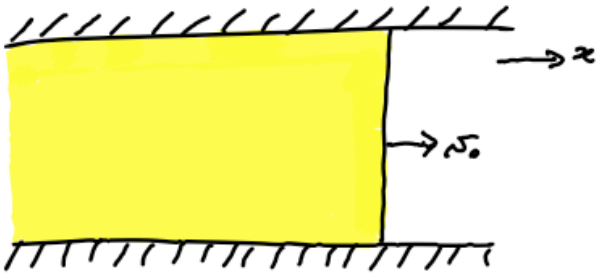


**Figure 1.5:** Two-dimensional proliferating tissue, confined between two plates.

The cell density conservation equation reads

$$\partial_t \rho + \partial_x(\rho v_x) = (k_d - k_a)\rho \tag{1.12}$$

As discussed above, we assume that proliferation depends locally on the density of the tissue

$$k_d - k_a = -\frac{1}{\tau}\frac{\rho - \rho_H}{\rho_H} \tag{1.13}$$

with $\tau$ a typical timescale for cell division.

The constitutive equation for the stress in the tissue reads

$$\sigma_{xx} = -P_H - \chi\frac{\rho - \rho_H}{\rho_H} \tag{1.14}$$

where $P_H$ is the homeostatic pressure in the tissue and $\chi$ a bulk elastic modulus. The proliferative pressure $P_H$ is assumed to be uniform across the tissue.

Here we furthermore assume that the tissue feels a friction force when it flows on its substrate, and we characterize it with a friction coefficient $\xi$, leading to the following force balance equation

$$\partial_x \sigma_{xx} = \xi v_x \tag{1.15}$$

From now on, we restrict ourselves to small deviations from the homeostatic density $\rho_H$, such that $\rho = \rho_H + \delta\rho$, with $\delta\rho \ll \rho_H$. Linearizing the cell density conservation equation, we obtain

$$\partial_t \delta\rho + \rho_H \partial_x v_x = -\frac{1}{\tau}\delta\rho \tag{1.16}$$

We now rewrite this last equation in the referential of the moving boundary of constant velocity $v_0$ by introducing the variable $z = x - v_0 t$. This leads to the following equivalence $\partial_t \to -v_0\partial_z$ and $\partial_x \to \partial_z$, and the following equation in $z$

$$-v_0\partial_z\delta\rho + \rho_H(\partial_z v_z) = -\frac{1}{\tau}\delta\rho$$

The velocity field $v_z = v_x$ can be calculated from the force balance equation

$$-\frac{\chi}{\rho_H}\partial_x \delta\rho = \xi v_x \tag{1.17}$$

which yields the following new equation for the cell density

$$-v_0 \partial_z \delta\rho - \frac{\chi}{\xi}\left(\partial_z^2 \delta\rho\right) = -\frac{1}{\tau}\delta\rho$$

For a small boundary velocity $v_0$, this equation simplifies into

$$-\frac{\chi}{\xi}\partial_z^2 \delta\rho + \frac{1}{\tau}\delta\rho = 0 \tag{1.18}$$

We can identify a characteristic length

$$\lambda = \sqrt{\chi\tau/\xi}$$

, and using the fact that the stress at the boundary $z = 0$ should vanish and the cell density shall not diverge for $z \to -\infty$, the only possible solution has the following form

$$\delta\rho = Ce^{\frac{z}{\lambda}} \tag{1.19}$$

with $C$ a constant to determine. The stress follows as

$$\sigma_{xx} = -P_H - \chi\frac{C}{\rho_H}e^{z/\lambda},$$

and using $\sigma_{xx}(z = 0) = 0$, we get $C = -P_H\frac{\rho_H}{\chi}$. The velocity is therefore deduced as

$$\begin{aligned} v_x &= \frac{\partial_z \sigma_{xx}}{\xi} = -\frac{\chi\partial_z\delta\rho}{\xi\rho_H} = \frac{P_H}{\xi}\lambda e^{z/\lambda} && (1.20)\\ &= P_H\frac{1}{\sqrt{\xi\chi\tau}}e^{z/\lambda} && (1.21)\end{aligned}$$

Finally, this allows us to calculate the expansion velocity $v_0$

$$v_0 = v_x(z = 0) = P_H\frac{1}{\sqrt{\xi\chi\tau}} \tag{1.22}$$

This equation shows that the speed of expansion of the tissue is slowed down by the friction on the substrate, as expected, and by the tissue bulk modulus, as cell density increases. In this model, all the tissue growth happens in a boundary layer near the interface of length $\lambda = \sqrt{\chi\tau/\xi}$. Further away from this interface, the pressure relaxes to the homeostatic pressure and there is no net cell division, and therefore no growth.

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