

Chapter 4

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*.

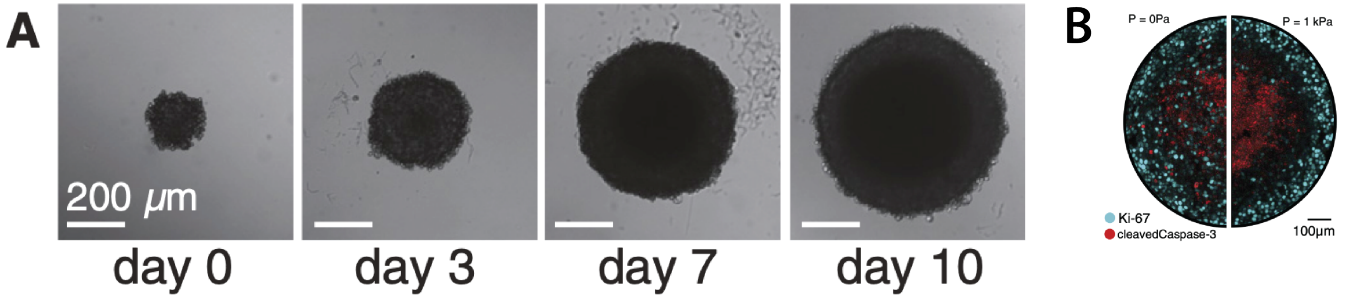


Figure 4.1: Growth of a cellular spheroid. (A) Snapshots of the time evolution of a spheroid made of CT26 mouse carcinoma cells [38]. (B) Distribution of proliferation and apoptosis in a spheroid (cyan corresponds to proliferation, red to apoptosis) in a tissue after 4 days of growth, with an isotropic stress of 1kPa for the right one [39].

4.1 Unconstrained growth of a spheroid

We start with simple considerations on a spherical spheroid.

4.1.1 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

$$\frac{\partial p_n}{\partial t} = k_d[(n-1)p_{n-1} - np_n] + k_a[(n+1)p_{n+1} - np_n] \quad (4.1)$$

Multiplying by n and summing on all n , we get

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

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 $\langle n \rangle$

$$\begin{aligned} \frac{\partial \langle n \rangle}{\partial t} &= k_d [\langle n(n+1) \rangle - \langle n^2 \rangle] + k_a [\langle n(n-1) \rangle - \langle n^2 \rangle] \\ &= (k_d - k_a) \langle n \rangle \end{aligned} \quad (4.2)$$

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

$$\begin{aligned} \frac{\partial \langle n^2 \rangle}{\partial t} &= k_d [\langle n(n+1)^2 \rangle - \langle n^3 \rangle] + k_a [\langle n(n-1)^2 \rangle - \langle n^3 \rangle] \\ &= 2 \langle n^2 \rangle (k_d - k_a) + (k_d + k_a) \langle n \rangle \end{aligned} \quad (4.3)$$

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

$$\begin{aligned} \frac{\partial \langle \delta n^2 \rangle}{\partial t} &= \frac{\partial \langle n^2 \rangle}{\partial t} - 2 \langle n \rangle \frac{\partial \langle n \rangle}{\partial t} \\ &= \langle n \rangle (k_d + k_a) + 2 \langle \delta n^2 \rangle (k_d - k_a) \end{aligned} \quad (4.4)$$

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

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

, such that δn 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 Langevin equation

$$\frac{dn}{dt} = (k_d - k_a)n + \sqrt{(k_d + k_a)n} \xi(t) \quad (4.5)$$

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 \mathbf{r} , we can similarly write an equation for the density $\rho(\mathbf{r}, t) \equiv \frac{n}{V}$, including the flux of cells \mathbf{r}

$$\frac{\partial \rho}{\partial t} + \nabla(\rho \mathbf{v}) = (k_d - k_a)\rho + \sqrt{\rho(k_d + k_a)} \xi(\mathbf{r}, t) \quad (4.6)$$

with

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

4.1.2 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 (4.6) reduces then simply to $\nabla \cdot \mathbf{v} = k_d - k_a \equiv k$, and even though 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 λ , and undergo apoptosis in the center, where the nutrient concentration is small, as depicted schematically on Fig. 4.2a.

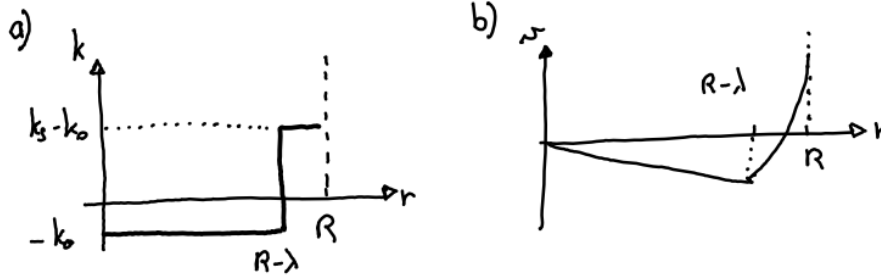


Figure 4.2: Growth of an incompressible spheroid. (a) Profile of the proliferation rate in the spheroid. (b) Radial velocity profile in the spheroid.

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. 4.2b.

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

$$v(R) = \frac{dR}{dt} = (k_s - k_0) \frac{R}{3} - k_s \frac{(R - \lambda)^3}{3R^2} \quad (4.7)$$

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

$$\frac{dN}{dt} = -k_0 N + k_s N_s, \quad \text{with} \quad N_s = n(V - V_\lambda) \quad (4.8)$$

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 λ .

- 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 $R = R_0 [1 - e^{-k_0 t/3}]$, 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) [39, 40], as shown on Figs. 4.3 and 4.4.

4.2 Coupling between stress and proliferation

4.2.1 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

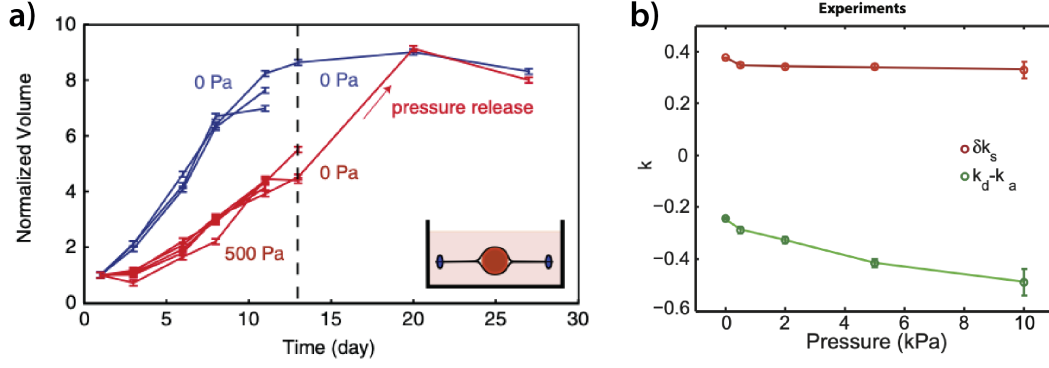


Figure 4.3: Growth of a cellular spheroid under stress [39]. (A) Time evolution of the volume of a spheroid with no stress and under stress. (B) Dependence of the rate of proliferation at the surface and in the bulk of a spheroid as function of the isotropic stress applied.

simple linear relationship

$$k_d - k_a = -\kappa \frac{\rho - \rho_H}{\rho_H} \quad (4.9)$$

where κ is an inverse timescale which quantifies the sensitivity of the tissue proliferation to local density and ρ_H is the density for which the division and apoptosis rates exactly balance each other, see Fig. 4.4a. 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.

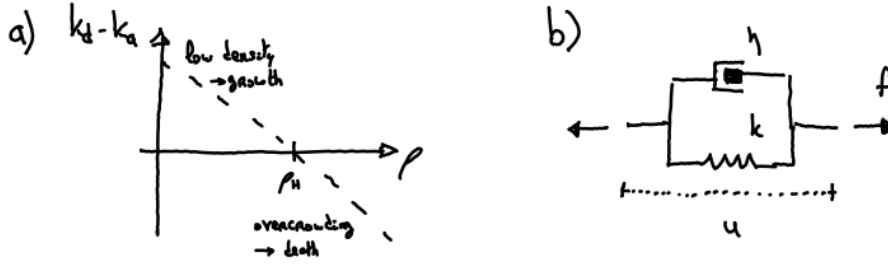


Figure 4.4: (a) Proliferation rate $k_d - k_a$ in the tissue as function of the local density ρ . (b) Rheological diagram of a Maxwell viscoelastic material of viscosity η and elasticity k .

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:

$$P = P_H + \chi \frac{\rho - \rho_H}{\rho_H} \quad (4.10)$$

where χ 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 (4.10) 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 [41].

Combining the two equations (4.9) and (4.10) with the cell density balance (4.6) (neglecting the noise term):

$$\frac{DP}{Dt} + \kappa \frac{\rho}{\rho_H} (P - P_H) = -\chi \frac{\rho}{\rho_H} \partial_k v_k \quad (4.11)$$

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 $(1 + \tau \frac{d}{dt}) = \eta \frac{du}{dt}$, of

viscoelastic timescale $\tau = \eta/k$, as depicted on Fig. 4.4b. 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 χ/κ .

4.2.2 Application: expansion of a proliferating confined tissue

As an application, we study here the expansion of a 2-dimensional proliferating tissue along the x -direction, confined in the y -direction between two plates, as depicted on the Fig. 4.5. It is supposed to have an infinite extension in the direction $x \rightarrow +\infty$, has a velocity v_x along x and a moving boundary of constant velocity v_0 .

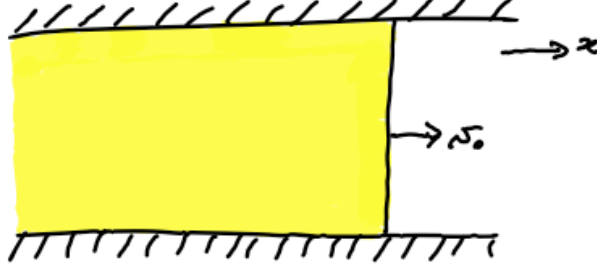


Figure 4.5: Two-dimensional proliferating tissue, confined between two plates.

The cell density conservation equation reads

$$\partial_t + \partial_x(\rho v_x) = (k_d - k_a)\rho \quad (4.12)$$

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

$$k_d - k_a = -\frac{1}{\tau} \frac{\rho - \rho_H}{\rho_H} \quad (4.13)$$

with τ a typical timescale for cell division.

The constitutive equation for the stress in the tissue reads

$$\sigma_{xx} = -P_H - \chi \frac{\rho - \rho_H}{\rho_H} \quad (4.14)$$

where P_H is the homeostatic pressure in the tissue and χ 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 ξ , leading to the following force balance equation

$$\partial_x \sigma_{xx} = \xi v_x \quad (4.15)$$

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

$$\partial_t \delta\rho + \rho_H \partial_x v_x = -\frac{1}{\tau} \delta\rho \quad (4.16)$$

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 $\partial_t \rightarrow -v_0 \partial_z$ and $\partial_x \rightarrow \partial_z$, 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

$$-\frac{\chi}{\rho_H}\partial_x\delta\rho = \xi v_x \quad (4.17)$$

which yields the following new equation for the cell density

$$-V_0\partial_z\delta\rho - \frac{\chi}{\xi}(\partial_z^2\delta\rho) = -\frac{1}{\tau}\delta\rho$$

For a small boundary velocity v_0 , this equation simplifies into

$$-\frac{\chi}{\xi}\partial_z^2\delta\rho + \frac{1}{\tau}\delta\rho = 0 \quad (4.18)$$

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 $z \rightarrow -\infty$, the only possible solution has the following form

$$\delta\rho = Ce^{\frac{z}{\lambda}} \quad (4.19)$$

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

$$v_x = \frac{\partial_z\sigma_{xx}}{\xi} = -\frac{\chi\partial_z\delta\rho}{\xi\rho_H} = \frac{P_H}{\xi}\lambda e^{z/\lambda} \quad (4.20)$$

$$= P_H\frac{1}{\sqrt{\xi\chi\tau}}e^{z/\lambda} \quad (4.21)$$

Finally, this allows us to calculate the expansion velocity v_0

$$v_0 = v_x(z = 0) = P_H\frac{1}{\sqrt{\xi\chi\tau}} \quad (4.22)$$

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.

Bibliography

- [1] Pierre-Gilles De Gennes, Françoise Brochard-Wyart, David Quéré, et al. *Capillarity and wetting phenomena: drops, bubbles, pearls, waves*, volume 336. Springer, 2004.
- [2] Malcolm S Steinberg. Reconstruction of tissues by dissociated cells. *Science*, 141(3579):401–408, 1963.
- [3] Philip L Townes and Johannes Holtfreter. Directed movements and selective adhesion of embryonic amphibian cells. *Journal of experimental zoology*, 128(1):53–120, 1955.
- [4] Ramsey A Foty, Cathie M Pfleger, Gabor Forgacs, and Malcolm S Steinberg. Surface tensions of embryonic tissues predict their mutual envelopment behavior. *Development*, 122(5):1611–1620, 1996.
- [5] Ramsey A Foty and Malcolm S Steinberg. The differential adhesion hypothesis: a direct evaluation. *Developmental biology*, 278(1):255–263, 2005.
- [6] Ramsey A Foty, Gabor Forgacs, Cathie M Pfleger, and Malcolm S Steinberg. Liquid properties of embryonic tissues: measurement of interfacial tensions. *Physical review letters*, 72(14):2298, 1994.
- [7] C Norotte, F Marga, A Neagu, I Kosztin, and G Forgacs. Experimental evaluation of apparent tissue surface tension based on the exact solution of the Laplace equation. *European Physical Letters*, 81(4):46003, January 2008.
- [8] JMt Mitchison and MM Swann. The mechanical properties of the cell surface. *J. exp. Biol*, 31(3):443–460, 1954.
- [9] Karine Guevorkian, Marie-Josée Colbert, Mélanie Durth, Sylvie Dufour, and Françoise Brochard-Wyart. Aspiration of biological viscoelastic drops. *Physical review letters*, 104(21):218101, 2010.
- [10] Francois Mazuel, Myriam Reffay, Vicard Du, Jean-Claude Bacri, Jean-Paul Rieu, and Claire Wilhelm. Magnetic flattening of stem-cell spheroids indicates a size-dependent elastocapillary transition. *Physical review letters*, 114(9):098105, 2015.
- [11] Jose E Perez, Irène Nagle, and Claire Wilhelm. Magnetic molding of tumor spheroids: emerging model for cancer screening. *Biofabrication*, 13(1):015018, 2020.
- [12] Albert K Harris. Is cell sorting caused by differences in the work of intercellular adhesion? a critique of the steinberg hypothesis. *Journal of theoretical biology*, 61(2):267–285, 1976.
- [13] François Graner and James A Glazier. Simulation of biological cell sorting using a two-dimensional extended potts model. *Physical review letters*, 69(13):2013, 1992.
- [14] G Wayne Brodland. The differential interfacial tension hypothesis (dith): a comprehensive theory for the self-rearrangement of embryonic cells and tissues. *J. Biomech. Eng.*, 124(2):188–197, 2002.
- [15] K Vijay Kumar. The actomyosin cortex of cells: A thin film of active matter. *Journal of the Indian Institute of Science*, 101(1):97–112, 2021.
- [16] Jean-Léon Maître and Carl-Philipp Heisenberg. Three functions of cadherins in cell adhesion. *Current Biology*, 23(14):R626–R633, 2013.

- [17] Jean-Léon Maître, Hélène Berthoumieux, Simon Frederik Gabriel Krens, Guillaume Salbreux, Frank Jülicher, Ewa Paluch, and Carl-Philipp Heisenberg. Adhesion functions in cell sorting by mechanically coupling the cortices of adhering cells. *science*, 338(6104):253–256, 2012.
- [18] M Lisa Manning, Ramsey A Foty, Malcolm S Steinberg, and Eva-Maria Schoetz. Coaction of intercellular adhesion and cortical tension specifies tissue surface tension. *Proceedings of the National Academy of Sciences*, 107(28):12517–12522, 2010.
- [19] Andrew R Harris, Loic Peter, Julien Bellis, Buzz Baum, Alexandre J Kabla, and Guillaume T Charras. Characterizing the mechanics of cultured cell monolayers. *Proceedings of the National Academy of Sciences*, 109(41):16449–16454, 2012.
- [20] Matthias Merkel, Raphaël Etournay, Marko Popović, Guillaume Salbreux, Suzanne Eaton, and Frank Jülicher. Triangles bridge the scales: Quantifying cellular contributions to tissue deformation. *Physical Review E*, 95(3):032401, 2017.
- [21] S Ishihara, K Sugimura, SJ Cox, Isabelle Bonnet, Y Bellaïche, and F Graner. Comparative study of non-invasive force and stress inference methods in tissue. *The European Physical Journal E*, 36(4):1–13, 2013.
- [22] Reza Farhadifar, Jens-Christian Röper, Benoit Aigouy, Suzanne Eaton, and Frank Jülicher. The influence of cell mechanics, cell-cell interactions, and proliferation on epithelial packing. *Current Biology*, 17(24):2095–2104, 2007.
- [23] Douglas B Staple, Reza Farhadifar, J-C Röper, Benoit Aigouy, Suzanne Eaton, and Frank Jülicher. Mechanics and remodelling of cell packings in epithelia. *The European Physical Journal E*, 33(2):117–127, 2010.
- [24] Edouard Hannezo, Jacques Prost, and Jean-Francois Joanny. Theory of epithelial sheet morphology in three dimensions. *Proceedings of the National Academy of Sciences*, 111(1):27–32, 2014.
- [25] Hisao Honda, Masaharu Tanemura, and Tatsuzo Nagai. A three-dimensional vertex dynamics cell model of space-filling polyhedra simulating cell behavior in a cell aggregate. *Journal of theoretical biology*, 226(4):439–453, 2004.
- [26] Satoru Okuda, Takashi Miura, Yasuhiro Inoue, Taiji Adachi, and Mototsugu Eiraku. Combining turing and 3d vertex models reproduces autonomous multicellular morphogenesis with undulation, tubulation, and branching. *Scientific reports*, 8(1):1–15, 2018.
- [27] Jean-Léon Maître, Hervé Turlier, Rukshala Illukkumbura, Björn Eismann, Ritsuya Niwayama, François Nédélec, and Takashi Hiiragi. Asymmetric division of contractile domains couples cell positioning and fate specification. *Nature*, 536(7616):344–348, 2016.
- [28] Jorge Nocedal and Stephen J Wright. Conjugate gradient methods. *Numerical optimization*, pages 101–134, 2006.
- [29] Sybren Ruurds De Groot and Peter Mazur. *Non-equilibrium thermodynamics*. Courier Corporation, 2013.
- [30] Karsten Kruse, Jean-Francois Joanny, Frank Jülicher, Jacques Prost, and Ken Sekimoto. Generic theory of active polar gels: a paradigm for cytoskeletal dynamics. *The European Physical Journal E*, 16(1):5–16, 2005.
- [31] Paul M Chaikin, Tom C Lubensky, and Thomas A Witten. *Principles of condensed matter physics*, volume 10. Cambridge university press Cambridge, 1995.
- [32] Vikram Deshpande, Antonio DeSimone, Robert McMeeking, and Pierre Recho. Chemo-mechanical model of a cell as a stochastic active gel. *Journal of the Mechanics and Physics of Solids*, 151:104381, 2021.

- [33] P Yo Chou. On velocity correlations and the solutions of the equations of turbulent fluctuation. *Quarterly of applied mathematics*, 3(1):38–54, 1945.
- [34] Pierre-Gilles De Gennes and Jacques Prost. *The physics of liquid crystals*. Number 83. Oxford university press, 1993.
- [35] S Fürthauer, M Stempel, Stephan W Grill, and Frank Jülicher. Active chiral fluids. *The European physical journal E*, 35(9):1–13, 2012.
- [36] G Duclos, C Blanch-Mercader, V Yashunsky, G Salbreux, J-F Joanny, J Prost, and Pascal Silberzan. Spontaneous shear flow in confined cellular nematics. *Nature physics*, 14(7):728–732, 2018.
- [37] Guillaume Duclos, Christoph Erlenkämper, Jean-François Joanny, and Pascal Silberzan. Topological defects in confined populations of spindle-shaped cells. *Nature Physics*, 13(1):58–62, 2017.
- [38] Morgan Delarue, Fabien Montel, Danijela Vignjevic, Jacques Prost, Jean-François Joanny, and Giovanni Cappelletto. Compressive stress inhibits proliferation in tumor spheroids through a volume limitation. *Biophysical journal*, 107(8):1821–1828, 2014.
- [39] Fabien Montel, Morgan Delarue, Jens Elgeti, Laurent Malaquin, Markus Basan, Thomas Risler, Bernard Cabane, Danijela Vignjevic, Jacques Prost, Giovanni Cappelletto, et al. Stress clamp experiments on multicellular tumor spheroids. *Physical review letters*, 107(18):188102, 2011.
- [40] Morgan Delarue, Fabien Montel, Ouriel Caen, Jens Elgeti, Jean-Michel Siaugue, Danijela Vignjevic, Jacques Prost, Jean-François Joanny, and Giovanni Cappelletto. Mechanical control of cell flow in multicellular spheroids. *Physical review letters*, 110(13):138103, 2013.
- [41] Jonas Ranft, Markus Basan, Jens Elgeti, Jean-François Joanny, Jacques Prost, and Frank Jülicher. Fluidization of tissues by cell division and apoptosis. *Proceedings of the National Academy of Sciences*, 107(49):20863–20868, 2010.