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Free boundary morphogenesis in living matter

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Abstract Morphogenetic theories investigate the creation and the emergence of form in living organisms. A novel approach for studying free boundary problems during morphogenesis is proposed in this work. The presence of mass fluxes inside a biological system is coupled with the local gradient of diffusing morphogens. The contour stability of a growing material is studied using a two-dimensional system model with rectilinear free border inside a Hele-Shaw cell. Modeling mass transport during morphogenesis allows fixing the velocity at the traveling wave solution as a function of one dimensionless parameter. Performing a perturbation of the free boundary, the dispersion relation is derived in an implicit form. Although both the velocity of the moving front and the surface tension act as stabilizing effects at small wavelengths, the dispersion diagrams show that the rectilinear border is always unstable at large wavelengths. Further applications of this model can help giving insights for a number of free boundary problems in biological systems.

Keywords Morphogenesis · free boundary growth · mass transport · living matter

1 Introduction

Morphogenesis can be defined as the branch of life sciences which investigates the creation and the emergence of form in living organisms. In a wide sense, morphogenetic processes result from both the genetic and the epigenetic information defining the complex schemes of matter differentiation [2]. Such

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regulation tasks interrelate the biochemical processes at the molecular level with the macroscopic variations of the environmental conditions. These coordination mechanisms give rise to the functional adaptation of living matter over an incredible variety of shapes and structures.
From a theoretical viewpoint much work has been done in the last decades to model the variation of mass and material properties in biological materials [1]. This highly interdisciplinary subject has involved a number of researchers with different scientific backgrounds, and many theoretical frameworks have been proposed. Nonlinear elastic theories have been successfully employed to describe how a geometrically incompatible volumetric growth can introduce residual stresses inside a biological material [17]. Using perturbation techniques it has been demonstrated that a bifurcation of the elastic equilibrium often occurs above a threshold *volumetric growth*, explaining the formation of various irregular shapes in soft tissues [7]. Mixture theory have proved to be more effective in problems where growth results from a mass exchange between the constituting phases, also taking into account the reaction-diffusion dynamics of biomolecules at the microscale [5]. Moreover, *discrete models are useful for describing systems of a relatively, numerically tractable, number of agents, irrespective of the specific domains* [9].
The investigation of free boundary shape changes in such models typically consist in solving an ordinary differential equation inside the living matter coupled with at least one reaction-diffusion equations for the biomolecules. Many mathematical studies have aimed at reproducing the complex patterns in living system by using phenomenological models with parabolic partial differential equations [14]. Other researchers have focused on the contour instability in growing materials using biologically motivated constitutive laws and imposing balance equations for thermodynamical consistency [4,3]. Although much work has been done to improve the accuracy and the resolution of morphogenetic theories, existing models are still too qualitative in nature for building valuable tools and establishing confidence in predicted outcomes [15].
A novel approach for studying free boundary problem during morphogenesis is proposed in the following. The theoretical model is defined in Sect. 2, coupling the mass fluxes inside a living system with the local gradient of diffusing morphogens. The theory is later applied to a two-dimensional system model with rectilinear frontier, whose traveling wave solution is given in Sect. 3. A linear stability analysis of the free boundary is presented in Sect. 4, deriving analytical conditions for the onset of a contour instability. Finally, I conclude and discuss the results of this work in Sect. 5.

2 Definition of the theoretical model

Let us consider a Hele-Shaw cell where a living material with a free rectilinear frontier (i.e. occupying a region $x \leq x_b$) is immersed into *a spatial domain occupied by an inviscid fluid*, as depicted in Figure 1 (a). A morphogenetic signal may diffuse inside the cell, *having an infinite reservoir at* $x \to \infty$*, which is not*

modified by the observed local dynamics (Figure 1, b). It is also known that the living matter possesses specific surface receptors which capture the morphogen at a typical uptake rate γ_n. Thus, indicating with n the concentration of the morphogen, the reaction-diffusion equations inside the Hele-Shaw cell read:

$$\dot{n}(x,y,t) = \begin{cases} D_n \Delta n - \gamma_n n & if \quad x \leq x_b \\ D_n \Delta n & if \quad x > x_b \end{cases} \tag{1}$$

where D_n is the diffusion coefficient inside the cell.

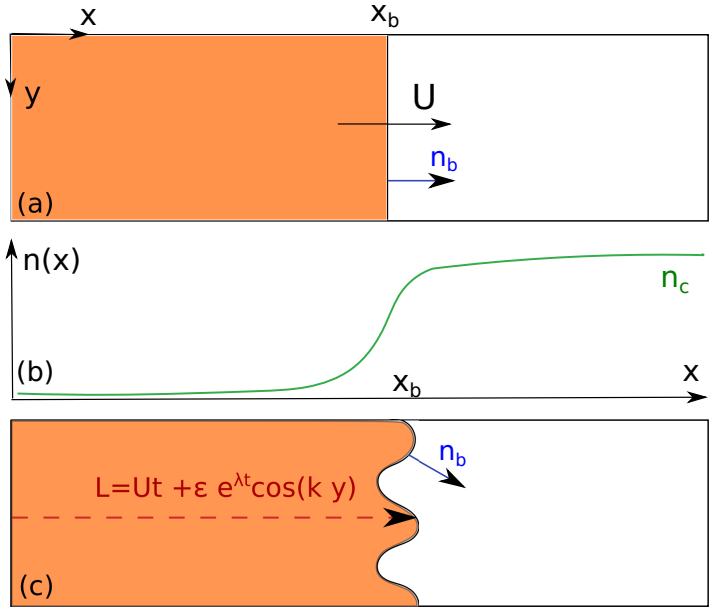

Fig. 1 Schematics of the Hele Shaw cell containing a living material (orange) expanding with a rectilinear free border at $x = x_b$ (a). Distribution of the morphogen concentration n (green line) inside the cell (b). Perturbation of the rectilinear border with wavenumber k and growth rate λ (c).

It is now widely accepted in developmental biology that morphogenesis resides on both diffusion and transport mechanisms of morphogens, acting not only as patterning agents but also as growth factors [12]. In the following, we consider that the growth of the living matter is driven by the gradient of the morphogen concentration. In particular, using the thermodynamical arguments considered in [6] under quasi-static conditions, a constitutive law for the spatial mass flux $\mathbf{m}$ is given as:

$$\mathbf{m} = \mathbb{K}^+(n)\nabla\Psi(n) = \frac{\partial\Psi(n)}{\partial n}\mathbb{K}^+(n)\nabla n \tag{2}$$

where $\Psi(n)$ is the free energy of the morphogen and $\mathbb{K}^+(n)$ is a positive definite mobility tensor, whose functional dependence on n may represent different

classes of biochemical reactions. According to Eq.(2), the balance of mass inside the biological material reads:

$$\frac{d\rho}{dt} = \nabla \cdot \ \mathbf{m} - \rho \ \nabla \cdot \mathbf{v} = \mathbb{K}^{+}(n)\nabla^2\Psi(n) + \nabla\mathbb{K}^{+}(n) \cdot \nabla\Psi(n) - \rho \ \nabla \cdot \mathbf{v} \quad (3)$$

where $\mathbf{v}$ is the physical velocity field and ρ is the spatial density. In Eq.(3) we considered that the morphogenesis only resides on mass diffusion, neglecting the presence of volumetric sources of mass.
Considering a very slow growth process and assuming that living matter macroscopically behaves as newtonian liquid moving at low Reynolds numbers, the mechanical equilibrium inside the Hele-Shaw cell is classically given by a Darcy law [11], being:

$$\mathbf{v} = -K_p \nabla p \quad (4)$$

where K_p is the porosity coefficient of the material, and p is the hydrostatic pressure. Substituting Eq.(4) in Eq.(3), the mass balance equation can be rewritten as:

$$\nabla \cdot \ \mathbf{m} - \rho \ \nabla \cdot \mathbf{v} = \nabla \cdot \left(\frac{\partial \Psi(n)}{\partial n} \mathbb{K}^{+}(n) \nabla n \right) + \rho K_p \ \Delta p = 0 \quad (5)$$

where we assumed that the biological matter is incompressible, being mostly composed by water. Eqs.(1,5) govern the morphogenesis of the free boundary domain in this model. For mathematical consistency, four boundary conditions at the free border must be imposed. The Young-Laplace equation imposes the mechanical equilibrium at the interface, being:

$$p = p_0 - \sigma C \qquad at \quad x = x_b \quad (6)$$

where C is the local curvature (*being equal to zero for a planar front*), σ is the surface tension *and* p_0 *is the constant outer pressure imposed inside the Hele-Shaw cell*. Moreover, a compatibility condition imposes the continuity of the normal velocity at the interface, which reads:

$$\frac{d\mathbf{x}_b}{dt} \cdot \mathbf{n}_b = \mathbf{v}(x_b) \cdot \mathbf{n}_b \qquad at \quad x = x_b \quad (7)$$

where $\mathbf{n}_b$ is the outward unit vector at the free surface. Finally, the two remaining conditions are derived assuming that there are no singular sources of morphogen, being:

$$n(x_b^-) = n(x_b^+); \qquad \nabla n(x_b^-) \cdot \mathbf{n}_b = \nabla n(x_b^+) \cdot \mathbf{n}_b \quad (8)$$

imposing the continuity of the morphogen concentration and of the chemical flux across the free boundary.

3 Dimensionless equations and their traveling-wave solution

In order to make an analytical study of the stability of the governing equations, let us assume a simple expression for the free energy of the morphogenetic signal and isotropic mobility tensor:

$$\Psi(n) = K_\Psi \cdot n; \qquad \mathbb{K}^+(n) = K_m \mathbf{I} \tag{9}$$

where K_Ψ, K_m are constant parameters representing the free energy per unit concentration and the motility coefficient of the morphogen, respectively.
Nondimensionalisation of the mathematical models involves the following dimensionless quantities:

$$\begin{gathered} t_c = \gamma_n^{-1}; \qquad L_c = \sqrt{\tfrac{D_n}{\gamma_n}}; \qquad v_c = \sqrt{D_n \gamma_n}; \\ p_c = \tfrac{D_n}{K_p}; \qquad n_c = n(x \to \infty) \end{gathered} \tag{10}$$

where t_c, L_c, v_c, p_c, n_c are characteristic time, length, velocity, pressure, and chemical concentration, respectively. Using such characteristic values to obtain dimensionless variables (hereafter indicated with barred symbols), we can rewrite the Eqs.(1,4, 5) in their dimensionless form, as follows:

$$\dot{\bar{n}}(R,t) = \begin{cases} \bar{\Delta}\bar{n} - \bar{n} & if \quad \bar{x} \le \bar{x}_b \\ \bar{\Delta}\bar{n} & if \quad \bar{x} > \bar{x}_b \end{cases} \tag{11}$$

$$\bar{\mathbf{v}} = -\bar{\nabla}\bar{p} \tag{12}$$

$$\bar{\Delta}\bar{p} = -B\bar{\Delta}\bar{n}; \quad \text{with} \qquad \mathrm{B} = \frac{\mathrm{K_m K_\Psi n_c}}{\rho \mathrm{D_n}} \tag{13}$$

Similarly, the boundary conditions in Eqs.(6-8) rewrite as:

$$\bar{p} = \bar{p}_0 - \bar{\sigma}\bar{C}; \quad \text{with} \qquad \bar{\sigma} = \frac{\sigma \mathrm{K_p}}{\mathrm{D_n}} \sqrt{\frac{\gamma_\mathrm{n}}{\mathrm{D_n}}} \quad \text{at} \quad \mathrm{x = x_b} \tag{14}$$

$$\frac{d\bar{\mathbf{x}}_b}{d\bar{t}} \cdot \mathbf{n}_b = \bar{\mathbf{v}}(x_b) \cdot \mathbf{n}_b \qquad at \quad x = x_b \tag{15}$$

$$\bar{n}(\bar{x}_b^-) = \bar{n}(\bar{x}_b^+); \qquad \bar{\nabla}\bar{n}(\bar{x}_b^-) \cdot \mathbf{n}_b = \bar{\nabla}\bar{n}(\bar{x}_b^+) \cdot \mathbf{n}_b \tag{16}$$

The mathematical model given by Eqs.(11-16) is entirely governed by the dimensionless parameters B and $\bar{\sigma}$ defined in Eqs.(13, 14), which will control the stability properties of the free boundary during morphogenesis. In physical terms, B represents a ratio between morphogen-driven mass production and the mass evacuation rate driven by porosity, while $\bar{\sigma}$ gives a ratio between the surface tension and the hydrostatic pressure.
For matters of notation compactness, we'll deal with dimensionless variables in the following sections, dropping the bar in their notation.
First, let us investigate the existence of traveling wave solutions of the problem. For this purpose, we will indicate with U the dimensionless velocity of the interface going from left to right, i.e. towards the highest concentration of the

chemical signal. Considering a dimensionless variable $\zeta = x - Ut$, we can derive the reaction-diffusion dynamics by solving Eqs.(11,16), as :

$$n_0(\zeta) = \begin{cases} n_0(0) \cdot e^{\frac{-U+\sqrt{U^2+4}}{2}\zeta} & if \quad \zeta \leq 0 \\ 1 + (n_0(0) - 1) \cdot e^{-U\zeta} & if \quad \zeta > 0 \end{cases} \tag{17}$$

where $n(-\infty) = 0$, $n(\infty) = 1$ and $n_0(0) = \frac{2U}{U+\sqrt{U^2+4}}$ is the morphogen concentration at the free boundary. It is useful to remark that $n(0)$ must be positive definite, so that the boundary expands with $U > 0$. Similarly, we can find the traveling wave for the pressure field imposing in Eqs.(13, 14) that $p_0(0) = p_0$ and that $p(-\infty)$ must be bounded, being:

$$p_0(\zeta) = -B(n_0(\zeta) - n_0(0)) + p_0 \qquad if \quad \zeta \leq 0 \tag{18}$$

Finally, the Dirichlet condition in Eq.(15) at the free boundary fixes the velocity of the traveling wave, which reads:

$$U = \frac{B-1}{\sqrt{B}} \tag{19}$$

revealing that a traveling wave solution can appear if and only if $B > 1$.

4 Linear stability analysis

The aim of this section is to study the stability of the traveling wave solution given by Eqs.(17,18). Let $\zeta(0)$ be a perturbation of the free boundary position, expressed in function of dimensionless variables in the moving frame as:

$$\zeta(0) = x_b - Ut = \epsilon \cdot e^{\lambda t} cos(\kappa y) \tag{20}$$

where κ is the spatial wavenumber and λ is its growth rate, as depicted in Figure 1(c).
We can express the variations on p and n in the moving frame as follows:

$$p(x, y, t) = p_0(\zeta) + \epsilon \cdot p_1(\zeta) e^{\lambda t} cos(\kappa y) \tag{21}$$

$$n(x, y, t) = n_0(\zeta) + \epsilon \cdot n_1(\zeta) e^{\lambda t} cos(\kappa y) \tag{22}$$

where $p_0(Z), n_0(Z)$ are the solutions of the unperturbed traveling wave solution moving along x.
From Eq.(11), we can find the solution for $n_1(\zeta)$ imposing the continuity of the chemical concentration and its flux across the boundary:

$$n_1(\zeta) = \begin{cases} n_1(0) \cdot e^{\frac{-U+\sqrt{U^2+4(1+\lambda+\kappa^2)}}{2}\zeta} & if \quad \zeta \leq 0 \\ n_1(0) \cdot e^{\frac{-U-\sqrt{U^2+4(\lambda+\kappa^2)}}{2}\zeta} & if \quad \zeta > 0 \end{cases} \tag{23}$$

where:

$$n_1(0) = -\frac{4U \cdot (U + \sqrt{U^2+4})^{-1}}{\sqrt{U^2+4(1+\lambda+\kappa^2)} + \sqrt{U^2+4(\lambda+\kappa^2)}} \tag{24}$$

Substituting the solution for $n_1(\zeta)$ in Eq.(13), we can find the governing equation for the perturbed pressure field (defined for $\zeta \leq 0$) at first order:

$$p_1{}''(\zeta) - \kappa^2 p_1(\zeta) + \\ + Bn_1(0) \cdot \left[\left(\frac{-U+\sqrt{U^2+4(1+\lambda+\kappa^2)}}{2} \right)^2 - \kappa^2 \right] e^{\frac{-U+\sqrt{U^2+4(1+\lambda+\kappa^2)}}{2}\zeta} = 0 \tag{25}$$

where prime denotes derivative on ζ. Eq.(25) has the following general solution:

$$p_1(\zeta) = -Bn_1(0) e^{\frac{-U+\sqrt{U^2+4(1+\lambda+\kappa^2)}}{2}\zeta} + A \cdot e^{\kappa\zeta} \tag{26}$$

The boundary solution in Eq.(14) can be used for determining the value of the constant A in Eq.(26):

$$p_1(\epsilon \cdot e^{\lambda t} cos(\kappa y)) = p_0(0) + (p_0{}'(0) + p_1(0))\epsilon \cdot e^{\lambda t} = p_o + \bar{\sigma}\kappa^2 \epsilon \cdot e^{\lambda t} cos(\kappa y) \tag{27}$$

giving at first order:

$$A = \bar{\sigma}\kappa^2 + B[n_1(0) + n_0{}'(0)] = \bar{\sigma}\kappa^2 + U + Bn_1(0) \tag{28}$$

Finally, the dispersion equation can be derived from the boundary condition in Eq.(15), expressed at first order as:

$$-p_0{}''(0) - p_1{}'(0) = \lambda \tag{29}$$

which can be simplified as:

$$\lambda = -\bar{\sigma}\kappa^3 - \kappa U + B \left[n_0{}''(0) + n_1(0) \cdot \left(-\kappa + \frac{-U + \sqrt{U^2 + 4(1+\lambda+\kappa^2)}}{2} \right) \right] \tag{30}$$

Substituting Eqs.(19, 24) in Eq.(30), we can express the dispersion equation as a function of the two dimensionless parameters $B, \bar{\sigma}$ of the problem:

$$\lambda = -\bar{\sigma}\kappa^3 - \kappa \frac{B-1}{\sqrt{B}} + \frac{B-1}{B} - \frac{(B-1) \cdot \left(-2\kappa - \frac{B-1}{\sqrt{B}} + \sqrt{\frac{(B+1)^2}{B} + 4(\lambda+\kappa^2)} \right)}{\sqrt{\frac{(B-1)^2}{B} + 4(\lambda+\kappa^2)} + \sqrt{\frac{(B+1)^2}{B} + 4(\lambda+\kappa^2)}} \tag{31}$$

The dispersion relation in Eq.(31) involves the dimensionless growth mode λ and wavenumber κ in an implicit way. In Figures 2 and 3, we show the dispersion diagrams obtained through numerical iteration techniques for different values of the dimensionless parameters B and $\bar{\sigma}$. We find that both the surface tension and the boundary velocity are stabilizing effects at small wavelengths, but the free boundary is always unstable at large wavelengths, developing undulated structures with typical length of about $2\pi/\kappa(\lambda_{max}) \cdot \sqrt{D_n/\gamma_n}$.

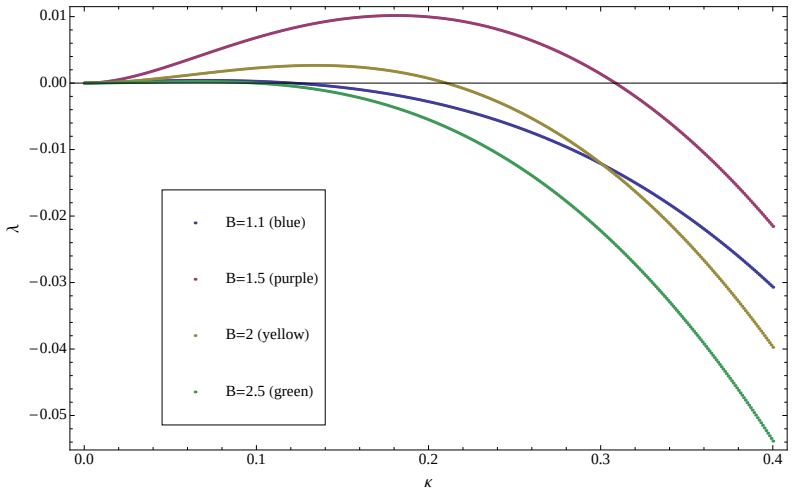

Fig. 2 Dispersion diagrams shown at different values of the dimensionless parameter $B = \frac{K_m K_y n_c}{\rho D_n}$. The curves have been numerically obtained using the Newton algorithm, setting σ=0.5.

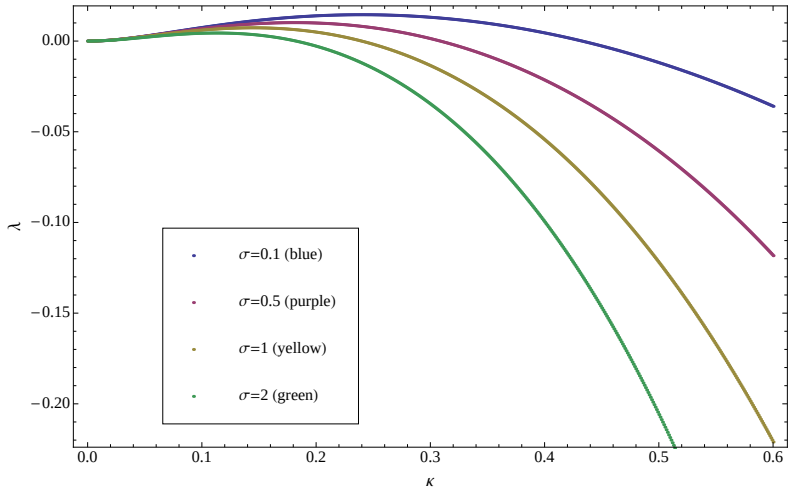

Fig. 3 Dispersion diagrams shown at different values of the dimensionless parameter $\bar{\sigma} = \frac{\sigma K_p}{D_n}\sqrt{\frac{\gamma_n}{D_n}}$. The curves have been numerically obtained using the Newton algorithm, setting B=1.5.

5 Discussion and conclusion

In this work, the contour stability of a growing living material has been studied using a two-dimensional system model inside a Hele-Shaw cell. In Sect. 2, a novel theoretical model of free boundary morphogenesis has been defined. In Eq.(2) the mass flux inside the living matter is assumed to depend on the local

gradient of a morphogen, whose dynamics is governed by the reaction-diffusion equations in Eq.(1). The biological material is considered as an incompressible newtonian fluid, so that the equilibrium equation in Eq.(4) is a Darcy law. The mass balance is imposed by Eq.(5), while the boundary conditions at the free rectilinear interface are given in Eqs.(6-8).
In Sect. 3, the governing equations have been rewritten in their dimensionless form and a traveling wave solution is given in Eqs.(17,18). In particular, the velocity of the moving front is fixed in Eq.(19) as a function of the dimensionless parameter B, defined in Eq.(13). It is interesting to notice that modeling mass transport in morphogenesis allows to overcome the problem to fix the velocity at the moving boundary, which keeps undetermined when considering only volumetric growth [10].
In Sect. 4, a linear stability analysis of the traveling wave solution has been reported. Performing a perturbation of the free boundary as in Eq.(20), an implicit relation has been derived in Eq.(31) as a function of the wavenumber κ and the growth rate λ of the perturbation. The dispersion diagrams have been numerically obtained as depicted in Figures 2 and 3.
Although both the velocity of the moving front and the surface tension act as stabilizing effects at small wavelengths, the dispersion diagrams show that the rectilinear border is always unstable at large wavelengths. Accordingly, the linear stability indicates that the rectilinear free boundary rapidly develops growing undulations. This result is in agreement with some experimental results on epithelial cells [16], showing the formation of fingering instabilities in expanding free rectilinear surfaces of cellular monolayers. *Further applications of this model can be envisaged for studying the morphology of self-organizing bacterial communities, whose swarming over a synthetic surface is found to create remarkable hyperbranched dendritic shapes [14].*

In conclusion, the application of the proposed model can help giving insights in a number of free boundary problems of biological systems. Typical examples concern the dynamics of wound healing processes, whose effectiveness depend on the coupling between motility and the cell proliferation rate [13], and the optimization of tissue scaffolds, where the feedback between growth rate and geometry must be deciphered [18]. Further work must be addressed for extending the analysis of the free boundary instabilities in a non-linear regime. In this sense, the development of simulation tools might be useful to study the occurrence of branching phenomena, such those observed during the differentiation of many developing organs [8].

References

1. Ambrosi D, Athesian GA, Arruda EM, Cowin SC, Dumais J, Goriely A, Holzapfel GA, Humphrey JD, Kemkemer R, Kuhl E, Olberding JE, Taber LA, Garikipati K (2011) Perspectives on growth and remodeling. J. Mech. Phys. Solids 59:863-883
2. Bard J (1990) Morphogenesis. Cambridge University Press, Dev. Cell. Biol. Ser. Vol. 23, Cambridge, UK.
3. Ben Amar M, Chatelain C, Ciarletta P (2011) Contour instabilities in early tumour growth models, Phys. Rev. Lett. 106(14): 148101.

4. Byrne HM, and Chaplain MAJ (1996) Free boundary value problems associated with the growth and the development of multicellular spheroids. Eur. J. Appl. Math. 8: 638-658.
5. Byrne HM, and Preziosi L (2004) Modelling solid tumour growth using the theory of mixtures, Math. Med. Biol. 20: 341-366.
6. Ciarletta P, Ambrosi D, Maugin GA (2012) Mass transport in morphogenetic processes: a second gradient theory for volumetric growth and material remodelling. J. Mech. Phys. Solids 60(3): 432-450.
7. Ciarletta P, and Ben Amar M (2012) Pattern formation in fiber-reinforced tubular tissues: folding and segmentation during epithelial growth. J. Mech. Phys. Solids 60(3): 525-537.
8. Davies JA (2006) Branching morphogenesis. Springer Science, New York.
9. Drasdo D, and Höeme S (2005) A single-cell-based model of tumour growth in vitro: monolayers and sphreroids. Phys. Biol. 2: 133-147.
10. Hartmann D, Miura T, (2007) Mathematical analysis of a free boundary model for lung branching morphogenesis. Math. Med. Biol. 24: 209-224.
11. Klapper I, (2004) Effect of heterogeneous structure in mechanically unstressed biofilms on overall growth. Bull. Math. Biol. 66: 809-824.
12. Lecuit T, and Lenne PF (2007) Cell surface mechanics and the control of cell shape, tissue patterns and morphogenesis. Nature Rev. Mol. Cell. Biol. 8: 633-644.
13. Maini PK, McElwain S, Leavesly DI, (2004) Traveling wave model to interpret a wound-healing cell migration assay for human peritoneal mesothelial cells. Tissue Eng. 10(3/4): 475-482.
14. Marrocco A, Henry H, Holland IB, Plapp M, Seror SJ, Perthame B (2010) Models of self-organizing bacterial communities and comparisons with experimental observations, Math. Model. Nat. Phenom. 5(1):148-162.
15. Oden JT, Hawkins A, Prudhomme S (2010) General Diffuse-Interface Theories and an Approach to Predictive Tumor Growth Modeling, Math. Mod. and Meth. in Appl. Sci. 20(3): 477-517.
16. Poujade M, Grasland-Mongrain E, Hertzog A., Jouanneau J, Chavrier P, Ladoux G. Beguin A, Silberzan P, (2007) Collective migration of an epithelial monolayer in respoinse to a model wound. Proc. Natl. Acad. Sci. USA 104(41): 15988-15993.
17. Rodriguez EK, Hoger A, McCulloch AD (1994) Stress-dependent finite growth in soft elastic tissues. J Biomech. 27: 455-467.
18. Rumpler M, Woesz A, Dunlop JW, van Dongen JT, Fratzl P (2008) The effect of geometry on three-dimensional tissue growth. J. R. Soc. Interface 5: 1173-1180.