Chemotactic collapse and mesenchymal morphogenesis

Carlos Escudero
Departamento de Física Fundamental, Universidad Nacional de Educación a Distancia, C/Senda del Rey 9, 28040 Madrid, Spain
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We study the effect of chemotactic signaling among mesenchymal cells. We show that the particular physiology of the mesenchymal cells allows one-dimensional collapse in contrast to the case of bacteria, and that the mesenchymal morphogenesis represents thus a more complex type of pattern formation than those found in bacterial colonies. We compare our theoretical predictions with recent *in vitro* experiments.

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I. INTRODUCTION

The development of spatial patterns is one of the most important topics in embryology. The formation of structure in embryology is known as morphogenesis. Although genes play a crucial role in the control of pattern formation, the importance of the mechanochemical interactions among the cells and their environment has been recognized in several works [1,2]. One of the advantages of this approach is that it has the potential for self-correction in contrast to the Turing chemical prepattern approach. Embryonic development is usually a very stable process, with the embryo capable of adjusting to many external disturbances. The prepattern approach implies the existence of potentially unstable processes and makes it difficult for the embryo to make the necessary adjustement to such disturbances as development proceeds [2].

In this work, we are concerned with one type of early embryonic cells known as dermal or mesenchymal cells, responsible for the formation of highly organized patterns on skin such as the primordia, which become feathers and scales, and the condensation of cells which mirror the cartilage pattern in developing limbs. Mesenchymal cells are capable of independent movement, due to long fingerlike protusions called filodopia which grab onto adhesive sites and pull themselves along: Spatial aggregation patterns in these appear as spatial variations in cell number density [3,4]. These cells can also secrete fibrous material which helps to make up the extracellular matrix tissue within which the cells move. However, experimental evidence indicates that there is not such a secretion during chondrogenesis and pattern formation of skin organ primordia [5], so we will neglect this contribution to the dynamics.

Here, we will analyze the role of chemotaxis in mesenchymal morphogenesis. It is known that chemotactic signaling is one of the most important mechanisms that lead to pattern formation in bacterial colonies [6], suggesting that its role might be crucial in morphogenesis. Actually, the presence of a powerful chemoattractant has been identified as one of the active agents of pattern formation in mesenchymal self-organization [7]. Probably, the simplest mathematical model for chemotactic aggregation is the Keller-Segel model [8]

$$\partial_t \rho = D_b \nabla^2 \rho - \nabla (k\rho \nabla c), \tag{1a}$$

$$\partial_t c = D_c \nabla^2 c + \alpha \rho. \tag{1b}$$

Here D_b is the cellular diffusion constant, k the chemotactic coefficient, α the rate of attractant production, and D_c the chemical diffusion constant. The terms in Eq. (1a) include the diffusion of the cells and chemotactic drift. Equation (1b) expresses the diffusion and production of attractant. Nondimensionalizing the systems (1a) and (1b) we get

$$\partial_t \rho = \nabla^2 \rho - \nabla (\rho \nabla c), \tag{2a}$$

$$\epsilon \partial_t c = \nabla^2 c + \rho, \tag{2b}$$

where $\epsilon = D_b / D_c$. An efficient chemotactic communication implies that the diffusion of the cells is much slower than the attractant diffusion, which leads us to consider $\epsilon = 0$. We finally arrive at the following nonlinear partial differential system:

$$\partial_t \rho = \nabla^2 \rho - \nabla (\rho \nabla c), \tag{3a}$$

$$-\nabla^2 c = \rho - k_0, \tag{3b}$$

where $k_0 = |\Omega|^{-1} \int_\Omega \rho dx$, and Ω is the region of the space where the system is defined, and $|\Omega|$ is its volume. Note that the introduction of k_0 provides a solvability condition for Eqs. (3a) and (3b) in the case of no-flux boundary conditions [9]. This system is known to blow up in finite time for dimension $d \geq 2$, but all the solutions are regular for $d = 1$ [10]. This means that in a three-dimensional system, while collapse to infinite density lines and points can occur, collapse to an infinite density sheet is mathematically impossible. This fact crucially affects the patterns that can form [11,12]. Actually, both types of chemotactic collapse have been already observed in experiments performed with *Escherichia coli* [13,14].

In the case of mesenchymal cells, far more complex than a bacteria like *Escherichia coli*, the situation is more involved. The supposition of short-range diffusion (or simply diffusion), that applies well to dilute systems, is not, in general, sufficiently accurate in such systems in which the cell densities are relatively high. The long filopodia extended by the cells can sense density variations beyond their nearest neighbors and so we must include a nonlocal effect on diffusive dispersal since the cells sense more distant densities and so respond to neighboring averages as well [2].

II. LONG-RANGE DIFFUSION

Long-range diffusion was traditionally modeled by the inclusion of a biharmonic term of the form ∇^4. This comes from the known fact that

$$\nabla^2\rho \propto \frac{\langle\rho(\mathbf{x},t)\rangle - \rho(\mathbf{x},t)}{R^2}, \quad \text{as } R\to 0, \tag{4}$$

where $\langle\rho\rangle$ is the average density in a sphere of radius R about $\mathbf{x}$, that is

$$\langle\rho(\mathbf{x},t)\rangle = \frac{1}{|V|}\int_V \rho(\mathbf{x}+\mathbf{r},t)d\mathbf{r}, \tag{5}$$

where V is the sphere of radius R. Because $R\to 0$, this suggests in the one-dimensional case the following Taylor series expansion:

$$\rho(x+r) = \exp(r\partial_x)\rho(x) = \left[1 + \frac{1}{2}r^2\left(1 + \frac{r^2}{12}\partial_x^2 + \cdots\right)\partial_x^2 + r\left(1 + \frac{1}{6}r^2\partial_x^2 + \cdots\right)\partial_x\right]\rho(x). \tag{6}$$

In the case of diffusion in an isotropic medium, after integration and truncation after the fourth term we obtain

$$\frac{\langle\rho(x,t)\rangle - \rho(x,t)}{R^2} = (D_2\partial_x^2 + R^2D_4\partial_x^4)\rho + o(R^6), \tag{7}$$

where the average is performed over the closed interval $[-R,R]$. This way we get the following extended diffusion equation:

$$\partial_t\rho = (D_2\partial_x^2 + R^2D_4\partial_x^4)\rho, \tag{8}$$

with $D_2, D_4 > 0$. An initial value problem to Eq. (8) blows up in finite time; this is a consequence of the asymptotic character of the functional Taylor expansion (7). Physically, this means that the cells move randomly but up a cell density gradient, a fact that goes against experiment. Further, the next higher truncation leads to a better behaved equation, but the corresponding solution is negative somewhere as known from Pawula's theorem [15]. Also, any approximation beyond the second order leads to a nonphysical increase of the number of boundary conditions, so we have to conclude that this is not a proper way to generalize diffusion. We can solve this problem regrouping the terms in Eq. (7) in the manner of Padé to get [16–18]

$$\frac{\langle\rho(x,t)\rangle - \rho(x,t)}{R^2} \approx \frac{D_2\partial_x^2}{1 - R^2(D_4/D_2)\partial_x^2}, \quad R\to 0. \tag{9}$$

The resulting extended diffusion equation is then

$$\partial_t\rho = \frac{D_2\partial_x^2}{1 - R^2(D_4/D_2)\partial_x^2}\rho, \tag{10}$$

where the diffusive operator is to be interpreted in the Fourier transform sense

$$\left(\frac{D_2\partial_x^2}{1 - R^2(D_4/D_2)\partial_x^2}\rho\right)^{\wedge} = \frac{D_2(-k^2)}{1 - R^2(D_4/D_2)(-k^2)}\hat{\rho}. \tag{11}$$

Equation (10) seems to be a proper extension of the diffusive aproximation to the nonlocal case as shown in Ref. [16]. However, this operator was introduced in this reference to simulate random motion in a lattice, while in the present work we are concerned with an integral operator. Similar expansions of integral operators have been already performed in different contexts, such as a regularization of the Chapman-Enskog expansion of the linear Boltzmann collisional operator [17], or an extension of the free-energy functional in the van der Waals theory of liquids [18]. The main difference is that when dealing with an integral operator some moments may not exist, and thus we cannot in principle expand such an operator. However, there is a way to circumvent this difficulty if we redefine the nonlocal approximate operator as

$$\frac{D_2\partial_x^2}{1 - \epsilon^2\partial_x^2} \tag{12}$$

where ϵ^2 is the ratio between the zeroth- and the second-order moments (note that this keeps our basic assumption that $\epsilon^2 \propto R^2$). This way we get the same long wavelength behavior as that yielded by the full integral operator (see Ref. [18] for the development of the theory).

III. CHEMOTACTIC COLLAPSE

We will see that considering long-range diffusion in systems (3a) and (3b) will lead to a finite time singularity in $d=1$, that implies collapse to an infinite density sheet in a three-dimensional system and to an infinite density line in a two-dimensional system. We are going to show this fact analytically, since numerical calculations are extremely unstable for precisely computing the existence of blowups in partial differential equations [19].

Studying the system in a one-dimensional space seems to be the determination of the mathematical properties of a toy model, but actually it is not. We only need to consider the problem in a space of the number of dimensions corresponding to the simultaneously contracting dimensions in the experimental setting. This is so because all the additional dimensions can remain invariant during the temporal evolution, leading to the reduced description of the problem. This is, if we observe chemotactic collapse in a one-dimensional space, this means that the chemotactic collapse can occur in a three-dimensional space forcing one dimension to collapse while the other two remain invariant. We will thus consider the system

$$\partial_t\rho = \frac{\nabla^2}{1 - \epsilon^2\nabla^2}\rho - \nabla c\cdot\nabla\rho + \rho^2 - k_0\rho, \tag{13a}$$

$$-\nabla^2 c = \rho - k_0, \tag{13b}$$

in one spatial dimension. Here, ϵ is proportional to the mean radius of a cell and the natural boundary conditions are no-flux boundary conditions (with a long-range gradient in the case of ρ)

$$\partial_x c|_{\partial\Omega} = \left.\frac{\partial_x}{1 - \epsilon^2\partial_x^2}\rho\right|_{\partial\Omega} = 0, \tag{14}$$

where Ω is the closed interval $\Omega=[-L,L]$, and $\partial\Omega$ is its boundary. Note that integrating the equation

$$\partial_t \rho = \frac{\partial_x^2}{1-\epsilon^2\partial_x^2}\rho - \partial_x(\rho\partial_x c) \tag{15}$$

over Ω and applying the boundary conditions we get the conservation of the total mass of ρ (as it should be, since we are only considering movement of the cells) and thus the conservation in time of k_0. To clarify the notation, let us explicity write the norm of a function f belonging to a $L^p(\Omega)$ space, $1 \leqslant p < \infty$

$$\|f\|_{L^p(\Omega)} = \left(\int_\Omega |f|^p dx\right)^{1/p}. \tag{16}$$

From Eq. (13a) we get

$$\frac{d}{dt}\frac{1}{2}\|\rho(\cdot,t)\|^2_{L^2(\Omega)} = \int_\Omega \rho\rho_t dx = \int_\Omega \rho\frac{\partial_x^2}{1-\epsilon^2\partial_x^2}\rho dx - \int_\Omega \rho\partial_x c\partial_x\rho dx + \int_\Omega \rho^3 dx - k_0\int_\Omega \rho^2 dx. \tag{17}$$

Now, we are going to estimate all the terms appearing in the right-hand side of this equation.

Integrating by parts the second term on the right-hand side of Eq. (17)

$$\int_\Omega \rho\partial_x c\partial_x\rho dx = \rho^2\partial_x c|_{\partial\Omega} - \int_\Omega \partial_x\rho\partial_x c\rho dx - \int_\Omega \rho\partial_x^2 c\rho dx, \tag{18}$$

that implies

$$\int_\Omega \rho\partial_x c\partial_x\rho dx = -\frac{1}{2}\int_\Omega \rho^2\partial_x^2 c dx = \frac{1}{2}\int_\Omega \rho^3 dx - \frac{k_0}{2}\int_\Omega \rho^2 dx. \tag{19}$$

The first term in the right-hand side of Eq. (17) can be estimated as follows:

$$\int_\Omega \rho\frac{\partial_x^2}{1-\epsilon^2\partial_x^2}\rho dx \leqslant \left|\int_\Omega \rho\frac{\partial_x^2}{1-\epsilon^2\partial_x^2}\rho dx\right| \leqslant \int_\Omega \left|\rho\frac{\partial_x^2}{1-\epsilon^2\partial_x^2}\rho\right| dx \leqslant \|\rho\|_{L^2(\Omega)} \times \left\|\frac{\partial_x^2}{1-\epsilon^2\partial_x^2}\rho\right\|_{L^2(\Omega)}, \tag{20}$$

where we have used Hölder's inequality (see below). By performing the shift of variables $y = x/\epsilon$, we get

$$\left\|\frac{\partial_x^2}{1-\epsilon^2\partial_x^2}\rho\right\|_{L^2(\Omega)} = \frac{1}{\epsilon^{(3/2)}}\left\|\frac{\partial_y^2}{1-\partial_y^2}\rho\right\|_{L^2(\Omega/\epsilon)} \leqslant \frac{N}{\epsilon^{(3/2)}}\|\rho\|_{L^2(\Omega/\epsilon)}, \tag{21}$$

where $N = |\partial_y^2(1-\partial_y^2)^{-1}|$. Let us clarify this last step a bit more. We have used the fact that the operator $\nabla^2(1-\nabla^2)^{-1}$ is bounded on every L^p space, with $1 \leqslant p \leqslant \infty$. This means that we can be assured that $\|\nabla^2(1-\nabla^2)^{-1}f\|_{L^p(\Omega)} \leqslant N\|f\|_{L^p(\Omega)}$ for every f belonging to $L^p(\Omega)$ and a constant N that does not depend on f (and thus N is called the norm of the operator). This fact can be easily seen once one realizes that the Fourier transform of the operator $\nabla^2(1-\nabla^2)^{-1}$ is a bounded function of the wave vector, and a rigorous proof can be found in [20]. We can again shift variables $x = \epsilon y$ to get

$$\int_\Omega \rho\frac{\partial_x^2}{1-\epsilon^2\partial_x^2}\rho dx \leqslant \frac{N}{\epsilon^2}\|\rho\|^2_{L^2(\Omega)}. \tag{22}$$

Finally, we can conclude our estimate as follows:

$$\int_\Omega \rho\frac{\partial_x^2}{1-\epsilon^2\partial_x^2}\rho dx \geqslant -\left|\int_\Omega \rho\frac{\partial_x^2}{1-\epsilon^2\partial_x^2}\rho dx\right| \geqslant -\frac{N}{\epsilon^2}\|\rho\|^2_{L^2(\Omega)}. \tag{23}$$

Now we are going to estimate the third term in Eq. (17)

$$\int_\Omega \rho^3 dx = \|\rho\|^3_{L^3(\Omega)}. \tag{24}$$

Hölder's inequality reads (for a rigorous proof of Hölder's inequality see [21])

$$\int_\Omega |uv| dx \leqslant \|u\|_{L^p(\Omega)}\|v\|_{L^q(\Omega)}, \qquad 1 \leqslant p,q \leqslant \infty, \quad \frac{1}{p}+\frac{1}{q} = 1. \tag{25}$$

Choosing $v = 1$ we get

$$\int_\Omega |u| dx \leqslant C\|u\|_{L^p(\Omega)}, \tag{26}$$

where $C = |\Omega|^{1/q}$. With this estimate we can claim that

$$\|\rho\|^2_{L^2(\Omega)} = \int_\Omega \rho^2 dx \leqslant C\|\rho^2\|_{L^p(\Omega)} = C\left(\int_\Omega \rho^{2p} dx\right)^{(1/p)} = C\left(\int_\Omega \rho^3 dx\right)^{(2/3)} = C\|\rho\|^2_{L^3(\Omega)}, \tag{27}$$

where we have chosen $p = 3/2$ (and correspondingly $q = 3$). This implies that

$$\|\rho\|_{L^3(\Omega)} \geqslant D\|\rho\|_{L^2(\Omega)}, \tag{28}$$

where $D = |\Omega|^{-1/6}$. Therefore, we have the final estimate

$$\frac{d}{dt}\|\rho\|^2_{L^2(\Omega)} \geqslant A(\|\rho\|^2_{L^2(\Omega)})^{(3/2)} - B\|\rho\|^2_{L^2(\Omega)}, \tag{29}$$

where $A, B > 0$ are constants, $A = |\Omega|^{-1/2}$ and $B = 2N/\epsilon^2 + k_0$. We are thus going to study the dynamical system

$$\frac{dx}{dt} = Ax^{3/2} - Bx. \tag{30}$$

This system has two fixed points, $x = 0$ and $x = (B/A)^2 > 0$. A linear stability analysis reveals that the positive fixed point is linearly unstable, meaning that every initial condition $x_0 > (B/A)^2$ will stay above this value for all times. Further, we know that the solution will grow without bound in this case, so we can claim the existence of two constants $t_0 < \infty$ and $0 < C_0 < A$, such that $Ax^{3/2}(t) - Bx(t) > C_0 x^{3/2}(t)$ for every $t > t_0$. This implies that

$$\frac{d}{dt}\|\rho\|^2_{L^2(\Omega)} > C_0(\|\rho\|^2_{L^2(\Omega)})^{(3/2)} \tag{31}$$

for $t > t_0$, and for an adequate initial condition. Solving this equation gives

$$\|\rho(\cdot,t)\|^2_{L^2(\Omega)} > \frac{1}{\sqrt{\|\rho(\cdot,t_1)\|^{-1}_{L^2(\Omega)} - \frac{C_0}{2}t}} \tag{32}$$

for $t > t_1 > t_0$, and for an adequate initial condition. Every adequate initial condition must fulfill

$$\|\rho(\cdot,0)\|^2_{L^2(\Omega)} > \frac{4N^2}{\epsilon^4}|\Omega| + \frac{4N}{\epsilon^2}\|\rho(\cdot,0)\|_{L^1(\Omega)} + \frac{1}{|\Omega|}\|\rho(\cdot,0)\|^2_{L^1(\Omega)}, \tag{33}$$

such as, for instance, $\rho(x,0) = (x^2+\delta)^{-1/4}$ and δ small enough. Thus we are finally led to conclude that the system does blow up in finite time.

IV. CONCLUSIONS

It has been argued that each of the aggregates in a pattern corresponds to a density singularity in the hydrodynamic description of the cells [11]. Our analysis predicts a different way of pattern formation from the usual models of chemotactic aggregation. In particular, an initial diffusive band can form a singularity by collapsing only one of its dimensions to zero thickness. This type of one-dimensional collapse has been already empirically observed: *in vitro* experiments showed that mesenchymal cells are able to aggregate by collapsing only one of the dimensions of the culture into stripes [7]. After a few days, an initial homogeneous layer begins to develop spatial structure, the cells beginning to align with their neighbors to form "swirls." This might be related to the fact that the homogeneous layer is not the "adequate" initial condition that we derived in our theoretical analysis. They can in contrast aggregate by collapsing two spatial dimensions following a standard Keller-Segel mechanism. When the distribution of cells is driven far enough from the homogeneous distribution, the "adequate" initial condition is then achieved, and the culture of cells begins to aggregate by collapsing only one of its dimensions into ridges. Quantitative testing of the theory is possible since Eq. (32) suggests one type of critical behavior (chemotactic collapse has the structure of a dynamical phase transition from a statistical mechanical point of view) characterized by certain measurable quantities under the form of critical exponents. In particular, we have [22]

$$\max_x \rho(x,t) \geqslant \frac{\tilde{A}}{\sqrt{T^* - t}}, \tag{34}$$

for an adequate constant $\tilde{A}$ and a blow-up time T^*, which implies that the critical exponent of our theory obeys $\alpha \geqslant 1/2$. An additional experimentally testable prediction is given by Eq. (33), which implies the existence of an optimal system size $|\Omega| = \epsilon^2/(2N)\|\rho\|_{L^1(\Omega)}$. This strongly suggests that the width of the stripes increases linearly with the number of the cells making up the transversal dimension of the stripe.

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[1] J. D. Murray, P. K. Maini, and R. T. Tranquillo, Phys. Rep. **171**, 60 (1988).
[2] J. D. Murray, *Mathematical Biology*, 2nd ed. (Springer, New York, 1993), and references therein.
[3] A. K. Harris, D. Stopak, and D. Wild, Nature (London) **290**, 249 (1981).
[4] A. K. Harris, P. Ward, and D. Stopak, Science **208**, 177 (1980).
[5] J. R. Hinchliffe and D. R. Johnson, *The Development of the Vertebrate Limb* (Oxford, Clarendon Press, 1980).
[6] E. Ben-Jacob, I. Cohen, and H. Levine, Adv. Phys. **49**, 395 (2000), and references therein.
[7] A. Garfinkel, Y. Tintut, D. Petrasek, K. Boström, and L. L. Demer, Proc. Natl. Acad. Sci. U.S.A. **101**, 9247 (2004).
[8] E. F. Keller and L. A. Segel, J. Theor. Biol. **26**, 399 (1970).
[9] J. J. L. Velázquez, SIAM J. Appl. Math. **62**, 1581 (2002).
[10] T. Nagai, Adv. Math. Sci. Appl. **5**, 581 (1995).
[11] M. P. Brenner, L. Levitov, and E. O. Budrene, Biophys. J. **74**, 1677 (1995).
[12] M. D. Betterton and M. P. Brenner, Phys. Rev. E **64**, 061904 (2001).
[13] E. O. Budrene and H. C. Berg, Nature (London) **349**, 630 (1991).
[14] E. O. Budrene and H. C. Berg, Nature (London) **376**, 49 (1995).
[15] R. F. Pawula, Phys. Rev. **162**, 186 (1967).
[16] C. R. Doering, P. S. Hagan, and P. Rosenau, Phys. Rev. A **36**, 985 (1987).
[17] P. Rosenau, Phys. Rev. A **40**, 7193 (1989).
[18] P. Rosenau, Phys. Rev. A **39**, 6614 (1989).
[19] A. J. Majda and A. L. Bertozzi, *Vorticity and Incompressible Flow*, Cambridge Texts in Applied Mathematics (Cambridge University Press, Cambridge, England, 2002).
[20] E. M. Stein, *Singular Integrals and Differentiability Properties of Functions* (Princeton University Press, Princeton, New Jersey, 1970).
[21] L. C. Evans, *Partial Differential Equations* (American Mathematical Society, Providence, Rhode Island, 2002).
[22] This last inequality comes from the embedding $\|\rho\|_{L^2(\Omega)} \leqslant \|\rho\|_{L^\infty(\Omega)}|\Omega|^{1/2}$ of Lebesgue spaces defined on compact sets.