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Simulation of embryonic cell self-organization: A study of aggregates with different concentrations of cell types

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A three-dimensional lattice simulation of embryonic cell movement driven by differential adhesion mechanisms is presented. Lattice sites can represent cells or external medium and surface tensions are defined by a Potts model with differential adhesivity, where the simulation temperature T controls cell diffusivity. The simulation is evolved by a Metropolis algorithm. We find that varying the relative concentration of the cell types in an aggregate can affect the dynamics of sorting. Below a concentration threshold of the high adhesive cell type, c_t, (≈ 0.5), sorting evolves by formation and coalescence of clusters of these cells, while above c_t an energetically metastable state develops where the high adhesive cells form a layer that encloses an internal cluster of low adhesive cells. In this case, sorting is much slower and proceeds to completion by diffusion of the cells belonging to the cluster through the layer of high adhesive cells. [S1063-651X(99)51303-2]

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During morphogenesis many biological mechanisms cooperate to organize cells in space and generate the form of an organism. Some mechanisms are, for example, cell division, cell death, cell migration, induction, cell adhesion, etc. Development is an example of a complex system at work where these mechanisms act in an orchestrated way. A possible approach to the study of such a problem is the following: we choose a basic mechanism, investigate its capacity to describe natural pattern formation and if limitations are found we introduce, successively, other mechanisms. In our approach we choose as a basic mechanism cell adhesion [1].

Dissociated and reaggregated cells that are obtained from different embryonic tissues *in vitro* have the ability to sort and regenerate homotypic (like-cell) domains. This cell behavior suggests that it can be important for cells to recover, at least partially, their positional information *in vivo*. For a review, see [2].

Cell sorting has many features typical of mixtures of immiscible liquids, e.g., oil and water. When water is dispersed in oil in an agitated container, the two phases undergo processes including aggregation, segregation, rounding, etc. that separate spatially the two phases driven by minimization of the interfacial free energy. Such processes are also observed with aggregates of cells.

Since cells adhere because they have molecules on their surface specialized for this function, a similar energy minimization theory was proposed to explain cell sorting, the differential adhesion hypothesis (DAH) [3]. According to the theory, the motile capacity of cells combined with the minimization of the adhesion free energy associated with cell-cell and cell-medium interfaces, drive the organization of the aggregate. In the light of the DAH an aggregate of cells is a sort of liquid. Experiments have confirmed that aggregates of biological cells have surface tension with properties typical of the liquid state [4,5].

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Recently, we studied cell motion in aggregates of cells obtained from neural retina and pigmented retina tissues of the eyes of nine-day-old chick embryos. The two cell types were dissociated from the original tissues and randomly mixed to reaggregate *in vitro*. The concentration of pigmented retina cells in the mixtures was very low (one pigmented cell for about 10^4 neural retina cells) so that they were surrounded only by neural retina cells. The motion of a few randomly selected pigmented cells in this situation was recorded for several hours and the results of the quantitative analysis presented a time velocity correlation and power spectra of position versus time compatible with random (Brownian-like) motion. This suggested that cell motion during sorting is biased by the overall energy minimization supporting the DAH. For more details, see [6,7]. More recently, Rieu, Kataoka, and Sawada [8] performed a more extensive analysis of cell motion in aggregates of endodermal and ectodermal cells of *hydra viridissima* to find that isolated endodermal cells in the bulk of the aggregates move randomly and that long range signaling between cells is not present, giving additional support to our findings and to the DAH.

In this Rapid Communication we present a simple model that holds the features compatible with the DAH and the experimental observations of cell interactions during cell sorting. Additional results of the model will be presented elsewhere [9].

We define a spin (a site label), $\sigma(i,j,k)$, at each site, (i,j,k) of a three-dimensional lattice. The site label can represent a cell of a given type or medium and cells are considered as hard spheres similar to the model proposed by Goel and Rogers [10]. The energy, which we define below, is similar to the model proposed by Graner and Glazier [11] but is much simpler. The cell types are light cells of low surface tension and dark cells with high surface tension. Surface energies are defined from the following Potts energy:

$$H=\sum_{i,j} e_{\sigma_i,\sigma_j}(1-\delta_{\sigma_i,\sigma_j}). \tag{1}$$

e_{σ_i,σ_j} are the contact energies per unit area of interface and

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depend on the nature of the contact; cell-cell or cell-medium. We use a cubic lattice with $70\times70\times70$ total number of sites. The simulation is evolved by a Metropolis algorithm at fixed temperature, T. At each time step a lattice site (i,j,k) and one of its 26 first neighbors are chosen randomly (see the discussion below about the range of interaction). The dynamics of the simulation is as follows: an exchange is tried between a cell and one of its 26 first neighbors and occurs with the following probability P:

$$P(\sigma\rightarrow\sigma')=e^{-\Delta H/T}, \quad \text{if} \quad \Delta H>0$$

or

$$P(\sigma\rightarrow\sigma')=1, \quad \text{if} \quad \Delta H\leqslant 0, \tag{2}$$

where ΔH is the energy change in the system produced by the exchange. The unit of time, Monte Carlo step (MCS), is defined by the number of random attempts of exchange and is equal to the total number of sites of the lattice.

Lattice symmetry introduces nonbiological behavior in the evolution of the system; the main effect is energy anisotropy, which is well understood. Fortunately, this effect can be decreased by extending the range of interaction between spins [12]. Although neighbor exchange occurs only with the closest 26 surrounding cells, the energy interaction range is higher to decrease lattice anisotropy. In this simulation the interaction range between spins is defined as eighth-nearest neighbor on a cubic lattice, i.e., the closest 116 surrounding sites of a given site. Then, in the present model a cell interacts with other cells at distances between one and two cell diameters away, limited to 116 adhesion sites. In this way we ensure that our results are lattice symmetry independent. The range of the interaction between cells can be biologically interpreted as a way of simulating the number of binding sites (10^{2-3}) on the surface of real cells.

According to experimental evidence [6,8], in the absence of adhesion and chemical gradients, cell motion is random. Then, for simplicity, we model it *as if* it were thermally induced, and so the simulation temperature controls cell diffusivity in the aggregate. The biological scenario is as follows: consider an isolated pigmented retina cell immersed in an aggregate of neural retina cells, as performed in some experiments [6]; in our simulation, an exchange where $\Delta E=0$ has probability 1, Eq. (2). Then when a dark cell is surrounded only by light cells or vice versa, an exchange with a neighboring cell has $\Delta E=0$ and is always taken. Since a neighbor is chosen randomly, the cell performs a three-dimensional random walk. The order parameters used are the homotypic (same cell), heterotypic (unlike cell), and cell-medium interface areas normalized by their sum [11,13].

From Eq. (1) we can define the surface tensions of the model as a function of the contact energies [14]. For an aggregate of two cell types d and l in contact with medium M, the surface tensions are

$$\gamma_{dl}=e_{dl}-\frac{e_{dd}+e_{ll}}{2}, \tag{3}$$

$$\gamma_{dM}=e_{dM}-\frac{e_{dd}}{2}, \tag{4}$$

$$\gamma_{lM}=e_{lM}-\frac{e_{ll}}{2}, \tag{5}$$

where γ_{dl}, γ_{dM}, and γ_{lM} are, respectively, the surface tensions at dark-light, dark-medium, and light-medium interface. However, for Potts energy, Eq. (1), $e_{ii}=0$ and the surface tensions reduce to

$$\gamma_{dl}=e_{dl}, \tag{6}$$

$$\gamma_{dM}=e_{dM}, \tag{7}$$

$$\gamma_{lM}=e_{lM}. \tag{8}$$

Recently, Foty *et al.* [4,5] have measured surface tensions in five different chick embryonic tissues and verified that the values found predict the mutual envelopment behavior of the tissues. For liver and neural retina cells of five-day-old embryos they found 4.6 and 1.6 dyn/cm, respectively. When opposed together, an aggregate of neural retina cells envelop an aggregate of liver cells. We use these numerical values for setting the surface tensions in the simulation at the dark-medium and light-medium interfaces, respectively. Then, we define $\gamma_{dM}=4.6$ and $\gamma_{lM}=1.6$ a.u. There are no measurements of surface tensions at heterotypic interfaces. We then choose an arbitrary but realistic value, $e_{dl}=0.6$ a.u., in agreement with the inequality $\gamma_{dM}>\gamma_{lM}+\gamma_{dl}$, which defines the condition for envelopment of a dark aggregate by a light one [14].

The choice of the temperature for running the simulations was obtained from the condition that diffusion should be low enough (as it is for real cells) for an initially cubic aggregate of light cells to be able to round completely without being trapped in metastable states [7]. This yields $T\approx8$, just 10% of the critical temperature $T_c\approx80$ where the system undergoes a transition to a disordered phase [9].

In this work we present a study of the dynamics of sorting when the relative concentration of two cell types in an aggregate varies. For that we start from a spherical aggregate where the cell types (dark and light) are distributed randomly and the concentration of dark cells, c, in the aggregate may vary. The total number of cells is 113 081.

In Fig. 1 we present snapshots of the time evolution of an aggregate with $c=0.3$. Observe the formation of isolated clusters of dark cells. The average size of these clusters is a function of c, higher c, higher their size. These clusters eventually merge and the single cluster formed is rounded at about 20 000 MCS. In graph 1 we plot the time evolution of the fractional dark-light interface. The unit of time is the Monte Carlo step; see above. These features are characteristic of aggregates with $c<0.5$.

In Fig. 2 we can see the evolution of an aggregate with $c=0.7$. Observe the formation of a layer of dark cells enclosing a cluster of light cells [Fig. 2(b)]. We can explain the development of this metastable state as follows: the higher concentration and inward migration of dark cells on the periphery of the aggregate during the formation of the external light layer, forms a closed layer that partially blocks the outward migration of light cells in the interior of the aggregate. A closed metastable layer is never formed below $c=0.5$ since a minimum number of dark cells is required, leaving at

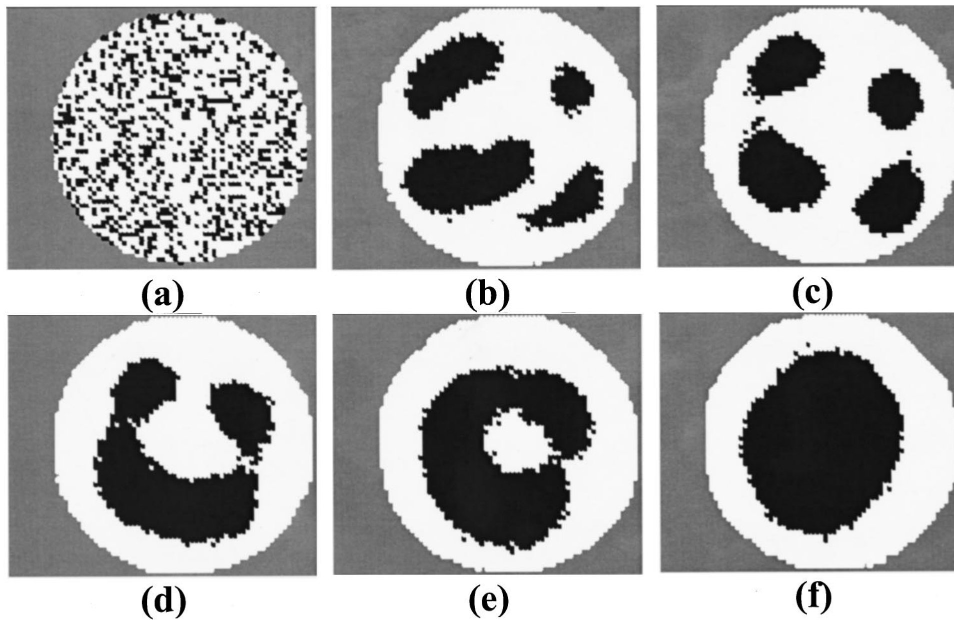

FIG. 1. Two-dimensional cross sections of the time evolution of an aggregate with $c = 0.3$. The unit of time is the Monte Carlo step (MCS); see text. (a) Initial state, (b) 2000 MCS, (c) 4000 MCS, (d) 9000 MCS, (e) 14 000 MCS, (f) 28 000 MCS.

least one opening for light cells to flow to the outside of the layer. In the presence of a closed layer ($c > 0.5$), light cells detach from the internal cluster and migrate diffusively to reach the external light layer [Fig. 2(c)]. In graph 1 we plot the time evolution of the fractional dark-light interface for this case. Its time evolution is much slower due to the formation of the internal light cluster that shrinks by cell diffusion. We have observed that the exponent for the process of shrinkage has a dependence on c. At about 275 000 MCS the internal core of dark cells is completely formed.

Aggregates with $c > 0.9$ sort as fast as aggregates with $c < 0.5$, as we can see in Figs. 3 and 4. This is reasonable since dark cells are predominant in the aggregate.

Finally, in Fig. 4 we plot the total time required for complete sorting as a function of the concentration of dark cells, c. It clearly shows the presence of a threshold value of $c_t \simeq 0.5$ that determines the formation of the closed layer of dark cells. This value is universal over the range of temperatures that we consider biological ($7 \simeq T \simeq 12$) and above it, i.e., when the cell types do not demix [9]. We are currently studying the effect of the surface tensions on c_t; preliminary results show that c_t has a dependence on γ_{dl} [9].

In conclusion, we have seen that the dynamics of sorting can have two phases, depending on the relative concentration of cell types in an aggregate: (i) when the concentration of dark cells is below c_t the total time needed for the process is

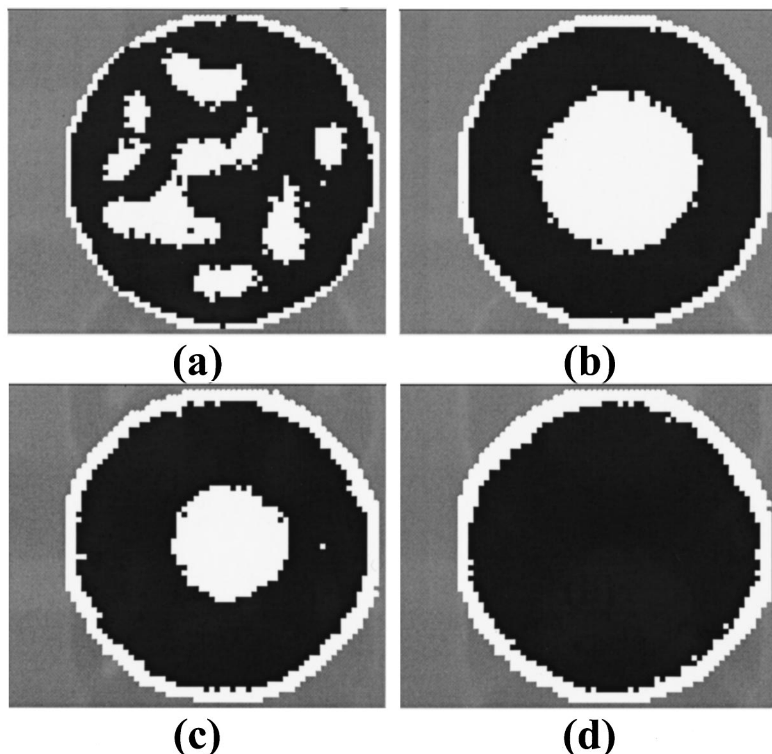

FIG. 2. Two-dimensional cross sections of the time evolution of an aggregate with $c = 0.7$. Initial state not shown. The unit of time is the Monte Carlo step (MCS). (a) 100 MCS. (b) 26 000 MCS. (c) 125 000 MCS. (d) 225 000 MCS.

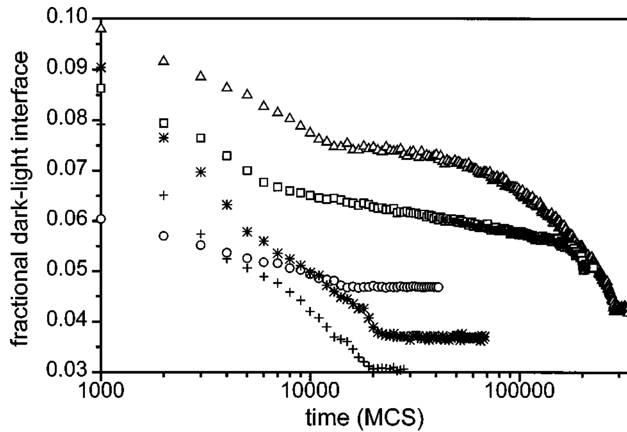

FIG. 3. Time evolution of the fractional interface between dark and light cells in the aggregates for $c=0.3$ (crosses), $c=0.4$ (stars), $c=0.5$ (triangles), $c=0.7$ (squares), and $c=0.9$ (circles). The unit of time is the Monte Carlo step (MCS).

weakly dependent on c (Fig. 4). Clusters of dark cells form and complete sorting occurs by encounter and coalescence of these clusters. (ii) For $c \geqslant c_t$, a metastable state develops, affecting the speed of sorting that changes abruptly, becoming much slower and strongly dependent on c (Fig. 4).

The results also suggest that different mitotic rates among cell types (whose immediate consequence is to change their

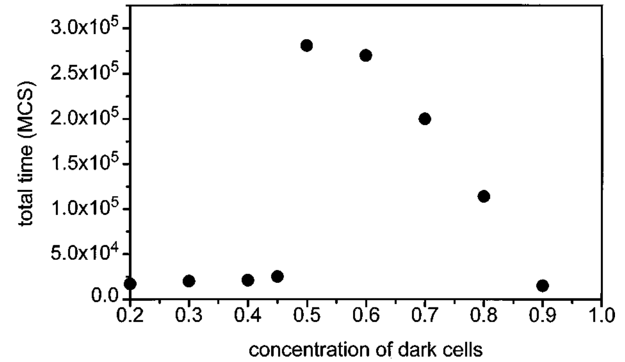

FIG. 4. Plot of the time needed for aggregates sort completely as a function of, c, the concentration of dark cells. The unit of time is the Monte Carlo step (MCS).

relative concentrations) can have substantial consequences during development. An experimental verification of this metastable state would yield additional support to the differential adhesion hypothesis.

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