Cell Clusters and Their Networks for Emerging Applications

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Abstract. Cell clusters and networks that interconnect them are key components of emerging applications such as 6G wireless molecular communication systems, drug delivery and tissue regeneration. This paper discusses empirical, analytical and simulation-based approaches towards understanding how spatially distributed cells aggregate to form cell clusters and how formed cell clusters grow and form a network that interconnects themselves. In order to illustrate these different approaches, this paper also describes authors' previous work relating to these approaches.

Keywords: Cell cluster · cell cluster network · cell spheroid · emerging application

1 Introduction

In emerging interdisciplinary applications such as 6G wireless molecular communication systems [1], drug delivery [2], tissue regeneration [3], bio-robotics [4], and organs-on-chips [5], biological cells and the clusters they form, as well as networks that interconnect cell clusters, are key components of the applications. A cell cluster is a three-dimensional aggregate of biological cells that is formed via cell-cell adhesion of individual cells. In applications, cell clusters communicate and coordinate with each other through exchanging molecules and perform application related functionalities.

In biology, spatially distributed cells exchange bio-chemical molecules, coordinate their movement, aggregate and form cell clusters. For instance, Dictyostelium discoideum cells exchange cAMP (cyclic adenosine monophosphate) molecules, move toward each other, and form clusters that rotate [6]. Endothelial cells exchange VEGF (vascular endothelial growth factor) molecules, move toward each other, and form cell clusters and a network that interconnects the clusters [7]. Cancer cells divide, increase in their numbers, and form cell clusters that perform collective invasion [8].

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In biomedical engineering, various techniques have been developed for the creation of cell clusters known as cell spheroids. The commonly employed technique utilizes a culture plate with a non-adhesive surface to prevent cells from adhering to the plate and to facilitate cell aggregation. Efforts are currently underway to develop techniques for establishing a network of cell spheroids. In [9], for instance, motor neuron spheroids are inter-connected through co-culturing with endothelial cells.

When cell clusters communicate and process information in a cooperative manner, it could help implement emerging interdisciplinary applications. For instance, drug delivery applications may benefit from having two types of the cell cluster in the environment and having them communicate. "Sensing" clusters detect specific bio-marker molecules in the environment, and "therapeutic" clusters produce therapeutic molecules. Upon detecting the presence of bio-marker molecules, sensing clusters send information to therapeutic clusters, which in turn produce therapeutic molecules.

The goal of this paper is to discuss empirical, analytic and simulation-based approaches to understanding how cells may form cell clusters and how cell clusters may form a network. In Sect. 2, we discuss empirical approaches and describe our preliminary wet laboratory experiments and demonstrate the feasibility of developing networks that interconnect spatially distributed cell clusters. We then discuss analytical approaches in Sect. 3 and simulation-based approaches in Sect. 4. Finally, in Sect. 5, we discuss future research challenges.

2 Wet Laboratory Experiments

An empirical (or experimental) approach enables observing, in the natural setting, how cells form a cluster and how such cell clusters form a network and communicate.

At the same time, the empirical approach presents its own challenges. For instance, although one can observe through biological experiments biological phenomena as they take place in a natural environment, it alone may not be sufficient to understand underlying mechanisms that cause what is being observed. Experimental protocols need to be established such that other researchers can reproduce experimental results. Types of cells available for biological experiments, i.e., types of cells whose basic behaviors have been experimentally established, may be limited. Many existing biological experiments are conducted in a 2D environment, and conducting biological experiments and observing experimental results in a more realistic 3D environment are not straightforward. Many studies report that cells behave differently in the 2D environment and 3D environment.

In the following, we briefly describe our empirical approach to understanding how cells form a cluster and how such cell clusters form a network.

To form a network of cell clusters, we first prepare cell clusters using EZSPHERE (AGC Techno Glass Inc., Shizuoka, Japan). We then transfer cell clusters to 35-mm glass-bottom dishes (Matsunami Glass, Kishiwada, Japan)

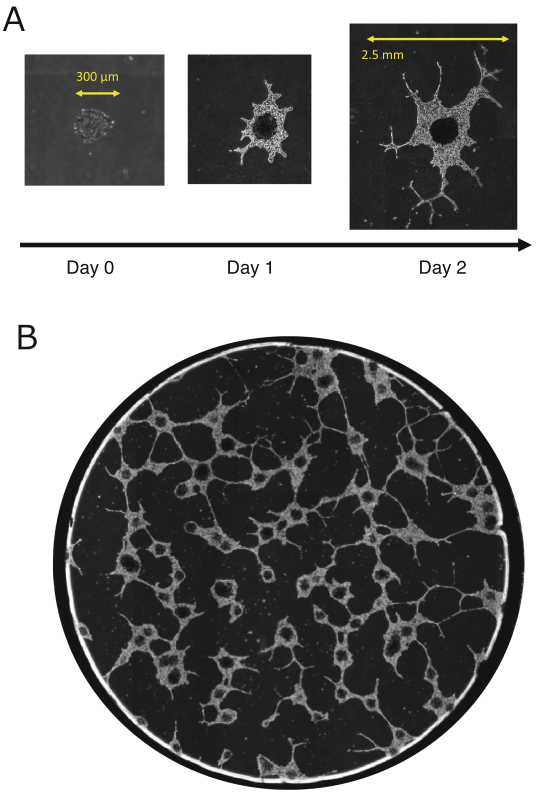

Fig. 1. Experimental results [10]. (A) A growing cell cluster at days 0, 1 and 2. (B) A cell cluster network interconnecting multiple cell clusters observed at day 3. The diameter of the circular area, 14 mm.

in Dulbecco's Modified Eagle Medium supplemented with 10% fetal calf serum where a layer of Matrigel (8–12 mg/mL; Falcon), a gelatinous protein mixture resembling the extracellular environment in tissue, is formed. We incubate the dishes at 37 °C under 5% CO_2 for 3–5 days, while we obtain phase contrast images every 24 h.

Figure 1 shows experimental results obtained in our previous work [10]. Figure 1(A) shows how a cell cluster grew. In this biological experiment, we first placed a (3D) spherical cell cluster of approximately 300 µm in diameter on Matrigel and observed how this cell cluster grew. As shown in the figure, the cell cluster grew as the cells inside the cluster moved, changed their morphology and divided. It grew to a size of about 2.5 mm while forming multiple needle-like arm structures over a period of 2 days.

Figure 1(B) shows a cell cluster network interconnecting multiple cell clusters. In this experiment, multiple cell clusters were first placed on Matrigel. We then observed that cell clusters constantly changed their shapes and extended multiple

needle-like arm structures; when these needle-like arm structures from different cell clusters encounter in the environment, they physically attach to each other and form a network that interconnects spatially distributed cell clusters.

3 Analytical Approaches

Analytical approaches allow one to focus on key system factors and ignore non critical factors, form and test a hypothesis regarding how cells form a cluster and how formed clusters communicate, and adjust the hypothesis based on the analytical results. Biological experiments should guide the choice of critical system factors to consider in the analytic approaches and hypothesis to form and test.

Analytical approaches may be either microscopic or macroscopic, depending on the level of abstraction. Microscopic models describe the state of each individual system entity. Macroscopic models describe the global state of the system. Microscopic and macroscopic models complement each other, as they describe dynamics at different levels.

3.1 Microscopic Modelling

Microscopic models describe the state of each individual system entity. For instance, using the Langevin equation [11], which was originally developed to describe the Brownian motion of a particle, movement of a system entity i (say, cell i) in a one-dimensional space may be described in the following manner.

$$m\frac{\mathrm{d}v_i}{\mathrm{d}t} = -\lambda v_i + \xi(t), \tag{1}$$

where cells move in a one-dimensional space, and the moving velocity v_i of cell $i \in \mathcal{N}$ is the variable of the equation and changes according to the above stochastic differential equation. On the left-hand side of (1), m is the mass of the cell, while on the right-hand side of (1), λ is a positive constant, and λv represents the resistance to the cell's motion. $\xi(t)$ represents a noise and follows a Gaussian probability distribution with zero mean $\langle \xi(t) \rangle = 0$ and correlation $\langle \xi(t_1) \cdot \xi(t_2) \rangle = 2\lambda k_B T \delta(t_1 - t_2)$, where k_B is Boltzmann's constant, T is the absolute temperature, and $\delta(\cdot)$ is the Dirac delta function.

3.2 Macroscopic Modelling

In contrast to the microscopic models described above, macroscopic models describe the global state of the system such as the number or concentration of cells. Macroscopic models usually take the form of a set of partial differential equations (PDEs).

Assume that cells move in a one-dimensional space, and let $c(x,t)$ be the concentration of cells at position x at time t. Using a diffusion equation, the rate of change in $c(x,t)$ is given by

$$\frac{\partial c(x,t)}{\partial t} = D\frac{\partial^2 c(x,t)}{\partial x^2} - \frac{\partial}{\partial x}\left[c(x,t)G(x)\right], \tag{2}$$

where D is the diffusion coefficient of cells, and G is a drift term. G can be used to describe the directed motion of cells. In the case of chemotactic bacteria, G becomes a function of attractant concentration and gradient of the attractant concentration [12] and is given as $G(x) \propto \chi(c_a)\frac{\partial c_a}{\partial x}$, where c_a is the attractant concentration at position x, and $\chi(c_a)$ is in the form of a Michaelis-Menten equation [13].

4 Simulation-Based Approaches

When a model increases its complexity, analytical solutions are often unavailable. In such cases, simulations-based approaches provide tractable alternatives.

A simulation is an imitation of the real-world process. Simulations require a model that represents the key behavior of the selected system element(s), and the simulation numerically calculates the evolution of the model over time. To conduct simulations, the time in the model equations is discretized, and the continuous-time model equations such as (1) and (2) become discrete-time difference equations, when necessary. Given initial conditions, then, the model equations are solved numerically to obtain the state of the selected system element(s) (e.g., the position of each and every cell or spatial cell distribution) at time t incrementally from time $t = 0$.

Simulation models can be classified into *off-lattice* and *on-lattice* models [14]: on-lattice models track cells along a rigid grid and off-lattice models have no such restriction. An agent-based model (ABM) [10] is an example of the former, and the Cellular Potts Model (CPM) is an example of the latter.

An Agent-Based Model. In the agent-based modelin our earlier study [10], each cell is an individual object and moves based on a set of forces it receives. In cells forming a cluster, we consider two types of force that acts on the cells: the attractive force and the repulsive force. Both the attractive force and the repulsive force acts between cells. The attractive force acts in a long-range, allowing cells at distance to move toward each other. The repulsive force acts in a short-range, allowing cells in their close proximity to move away from each other. The two types of force determine the size of the cluster that cells form.

In the wet-laboratory experiments in our earlier study shown in Fig. 1(A), some cells at the periphery of a cluster moved away from the cluster and other nearby cells followed the cells moving away from the cluster; as a result, the cluster grew multiple branches. In collective cell migration [15,16], such cells moving away from the cluster they belong to are called *leaders*, while others are

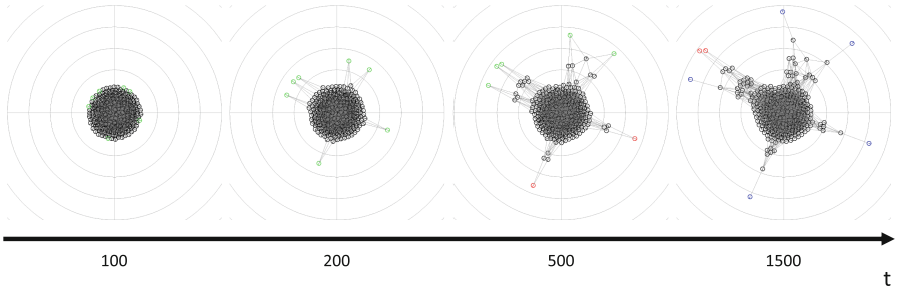

Fig. 2. Time evolution of a cell cluster in the agent-based simulation [10]

called *followers*. Leaders have free edges or more extensive substrate interactions than followers. In our model [10], cell i acts as a leader when it is in physical contact with a small number of cells at time t; otherwise, cell i acts as a follower. Leaders and followers use different types of force to move: the forward force and following force, respectively.

Figure 2 shows a simulation result obtained using our agent model. At time $t = 0$, 500 cells formed a cluster. At time $t = 100$, cells differentiated into leaders (colored) and followers (gray). Leaders then move away from the cluster center while followers follow them so that a cluster grows and extends its branches (at time $t = 200$, 500 and 1500).

Cellular Pots Model (CPM). In another simulation-based study of ours, we employed the Cellular Pots Model (CPM). The CPM is a discrete-time simulation model that represents cells or cell population on a regular and orthogonal grid [17]. In CPM, each cell is assigned a numerical ID, and it is represented as a set of lattice sites with the same numerical ID, unlike agent-based models, which treat each cell as an individual object. CPM is suitable to reproduce complex cell shapes such as those in Fig. 1.

In CPM [17], a cell is represented by a collection of lattice sites. A cell with label N $(1 \leq N \leq M)$ is represented by a collection of all lattice sites to which a numerical ID of N is assigned (i.e., all lattice sites i for which $\sigma_i = N$). Here, M is the total number of cells.

In CPM, there is an energy E associated with a given arrangement of cells.

$$E = \sum_{i,j} J_{\sigma_i \sigma_j}(1 - \delta_{\sigma_i \sigma_j}) + \sum_{N=1}^{M} \kappa(A_N - A_0)^2 + \sum_{N=1}^{M} \gamma(L_N - L_0)^2 \tag{3}$$

Noting that $\delta_{\sigma_i \sigma_j}$ is the Kronecker delta, which takes the value of 1 if $\sigma_i = \sigma_j$ and 0 otherwise. The first term in the above equation represents the energy concerning the cell-cell interaction and is calculated only for the lattice sites

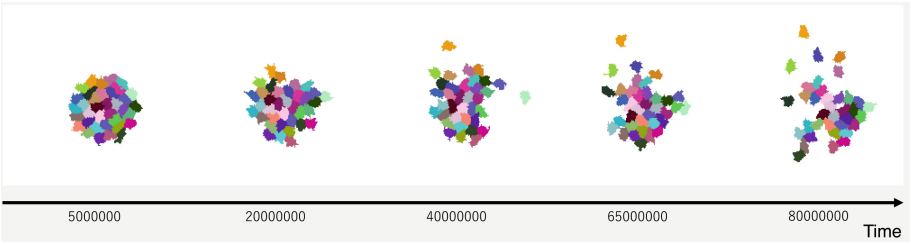

Fig. 3. Time evolution of a cell cluster in the CPM

located at the boundary of a cell to its adjacent cells. The second term and third term in the above equation represent the energy concerning the area elasticity and that concerning the perimeter elasticity of the cell, respectively. κ and γ in these terms are constants and determine the energy. A_N and L_N in these terms represent the area and perimeter of cell N. The substrate in which no cells reside is represented by lattice sites labeled with 0. A_0 and L_0 are the target area and target perimeter. The sum in the second and third terms is calculated for all cells on the lattice.

In simulations, we select a randomly chosen lattice site i. If it is located at the boundary of cell N, we select one of the lattice sites, say lattice site j, adjacent to site i. We calculate the difference ΔE between the energy before making the lattice site j identical to the lattice site i and after doing so, and compute the probability P_r of whether this change should take place using the following equation.

$$P_r = \begin{cases} 1 & (\Delta E \leq 0) \\ \exp\left(-\Delta E/T\right) & (\Delta E > 0) \end{cases} \tag{4}$$

The symbol T is a constant referred to as the temperature of the simulation in CPM and determines the transition probability P_r when $\Delta E > 0$.

Figure 3 shows the simulation result obtained using the CPM in our earlier study. In our earlier study, we extended the CPM in order to reproduce characteristics of cells observed in biological experiments. In our extended CPM model, cells at the periphery of the cluster probabilistically moved away from the cluster. Once cells started moving away from the cluster, they maintained the direction of movement, and other nearby cells followed them to form a narrow and long needle-like arm structures. This resulted in a cluster with a complex-shape as shown in the figure. Our extended CPM model reproduced some features of a cell cluster observed in wet-laboratory experiments in Fig. 1.

5 Future Work

One of the key future challenges is to identify how cell clusters and their networks may be used to implement emerging applications.

Applications that may benefit from cell clusters and the network that interconnects them include regenerative medicine, neural spheroid networks, and brain science. For instance, in the regenerative medicine application, cell clusters may communicate and coordinate their movement with other cell clusters to form a 3D structure of a given internal organ. In the neural spheroid network application, cell clusters (representing a group of neurons) may communicate and coordinate with other cell clusters through narrow needle-like links of cells. Neural spheroid networks may also be linked with networks comprising of other types of cells, such as endothelial cells, to establish an experimental model of the human brain to help study human brain functions.

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