Continuous models for cell-cell adhesion

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Abstract

Cell adhesion is the binding of a cell to another cell or to an extracellular matrix component. This process is essential in organ formation during embryonic development and in maintaining multicellular structure. Armstrong, Painter and Sherratt (2006)[J. Theor. Biol. 243, pp. 98–113] proposed a nonlocal advection-diffusion system as a possible continuous mathematical model for cell-cell adhesion. Although the system is attractive and challenging, it gives biologically unrealistic numerical solutions under certain situations. We identify the problems and change underlying idea of cell movement from “cells move randomly” to “cells move from high to low pressure regions”. Then we provide a modified continuous model for cell-cell adhesion. Numerical experiments illustrate that the modified model is able to replicate not only the Steinberg’s cell sorting experiments but also some phenomena which can not be captured at all by Armstrong-Painter-Sherratt model.

Keywords: Cell-cell adhesion, Cell sorting, Cell movement, Mathematical model, Diffusion, Advection

1. Introduction

Cell-cell contact and adhesion are a fundamental process in the development of multicellular organisms. Tissues and organs are formed by numerous cells, and these cells must keep in physical contact with others for their structural and functional communications. The cell-cell adhesion system is involved in many aspects of the development of multicellular organism, including not only adhesion but also movement, recognition, polarization, and cell shape change. Many of these steps require cell-cell interactions, and cell-cell contacts provide a platform for these cell-cell interacting processes. The cell-cell contact is mediated by transmembrane cell-adhesion molecules (CAMs), which bind similar partner proteins on opposing cells, or adhesion receptors, which bind various ligands. There are several classes of molecules that can mediate cell adhesion. The major cell adhesion molecules are the cadherins. Cadherins comprise a superfamily consisting of over one hundred members (see, e.g., Takeichi (2011)). The major role of cadherins is to connect cells expressing the same cadherins through their homophilic interactions. Cadherin activities are indispensable for firm and ordered cell-cell associations.

When the dispersed cells from two different vertebrate embryonic tissues are randomly intermixed in an aggregate, sorting out usually occurs (Armstrong (1971); Moscona (1957); Townes and Holtfreter (1955)) (Figure 1). Dissociated cells of embryos, which derived from different

Figure 1: Cell sorting in combinations of chick embryo neural retinal and pigmented retinal epithelial cells. Cell sorting experiments using seven-day chick embryo eye. These figures show that aggregations of dissociated neural retinal (colorless) and pigmented retinal cells (black). (a) 5 h old aggregate, two types of cell are randomly mixed in the aggregate. (b) 19 h old aggregates, the pigmented retinal cells are almost exclusively located in the interior of the aggregates. (c) 2-day old aggregates, the pigmented retinal cells have formed single internal mass, completely surrounded by the neural retinal cells. (From Armstrong (1971))

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germ layers, reaggregate and reorganize the tissue's specific structures through a process of segregating different types of cell populations. Townes and Holtfreter (1955) developed the concept of selective affinity from this finding. The inner surface of the ectoderm has a positive affinity for mesodermal cells and a negative affinity for the endoderm, while the mesoderm has positive affinities for both ectodermal and endodermal cells. Then cells expressing different cadherins are mixed together, they sort out and aggregate separately, indicating that different cadherins preferentially bind to their own type (Nose, Nagafuchi and Takeichi (1988)). For example, E-cadherin-expressing cells adhere to other E-cadherin-expressing cells, and N-cadherin-expressing cells adhere to those expressing N-cadherin. These phenomenon seems likely what happens when cells derived from different tissues are mixed together. These observations suggest that the differential adhesiveness of cadherin can be implicated in the cell sorting in morphogenesis.

Another view of cell sorting is based on quantitative differences in cell adhesiveness. More than 50 years ago, Steinberg proposed differential adhesion hypothesis to explain the force direct cell movement during morphogenesis (Steinberg (1962a,b,c,d)). The differential adhesion hypothesis explains patterns of cell sorting and cellular movement during morphogenesis with thermodynamic principles (Figure 2). Steinberg proposed that, by analogy with

Figure 2: Differential adhesion hypothesis. (a) Preferential cross adhesion. If the cross-adhesions between two types of cell populations are as strong as or stronger than the average of the two cell populations' individual cohesivities, the two cell populations should intermix. (b) Intermediate cross-adhesion. If the cross-adhesions are at least as strong as those between the less cohesive of the two cell populations but not as strong as the average of the two cell populations' individual cohesivities, the less cohesive cell population should envelop its partner completely. (c) Relatively low cross-adhesion. If the cross-adhesions between two types of cell populations could occur, but more weakly than identical type of cell population have a configuration of partial envelopment of one cell population by the other. (d) No cross-adhesion. If the two populations of cell could not cross-adhere, then otherwise non-cohesive cells should form separate spheres. (From Foty and Steinberg, 2004)

inanimate immiscible liquids whose rearrangements are driven by intermolecular attractions that act to maximize global binding energy, these rearrangements of cells are guided by intercellular adhesions acting to do the same (Foty and Steinberg (2004)). Such behaviour of cells was indeed proven by using a mixture of cells. In an aggregate containing a mixture of cells expressing same level

of cadherin, the two cell populations did not segregate. In contrast, in the aggregate containing a mixture of the cells expressing higher amounts of cadherin and those expressing lower amounts of cadherin, these two lines of cells segregate from one another, with the lower-expressing cells completely enveloping the higher-expressing cells (Duguay, Foty and Steinberg (2003); Steinberg and Takeichi (1994)). It therefore seems likely that both qualitative and quantitative differences in the expression of cadherins have a role in organizing tissues.

Cell sorting shapes the body plan but does not explain more intricate patterns seen at single cell level in tissues and organs. One such pattern is the checkerboard-like cell arrangement, which is observed in the auditory epithelia. Recently, one of the authors reported that the heterophilic interactions of nectins are critical for establishing the checkerboard-like cellular pattern formation (Togashi et al. (2011)). Nectins are immunoglobulin-like CAMs that compose a family of four members (nectin-1, -2, -3, and -4) (Takai and Nakanishi (2003)). Nectins prefer heterophilic interactions to homophilic ones, in contrast with the homophilic nature of cadherin interactions. For example, when cells expressing nectin-1 and -3 are mixed, these cells are arranged in a mosaic pattern due to their heterophilic interactions (Togashi et al. (2006, 2011)). Figure 3 shows experiments with cells expressing (a) nectin-1 and -1, (b) nectin-1 and -3. Before the time-lapse experiments, these cells were separately cultured. In early stages of cultures, they grow proliferously and expand their habitats. When their colony edges came into contact with one another, their boundaries were examined. Cells expressing identical nectin types did not overlap at later times. Cells expressing different nectin types were intermingling with one another after contact.

In this paper we consider mathematical models capable of replicating these types of cell adhesion experiments. A number of discrete models have been developed for cell sorting phenomena. Many of them are based on a framework the so called cellular Potts model. Those models are on lattice space. Each lattice site can be occupied by at most one cell. Each cell occupies many lattice sites and moves in response to its surroundings (e.g., Chen et al. (2007); Graner and Glazier (1992); Krieg et al. (2008); Maeda, Ajioka and Nakajima (2007)). Another approach uses single-cell-based lattice-free models. Each cell is represented by an individual object and characterized by its location in continuous space. They interact locally with their neighbours (e.g., Palsson and Othmer (2000); Sulsky, Childress and Percus (1984)). These single-cell-based models have been used to understand small-scale phenomena or situations and successfully replicated the cell sorting dynamics. However, solving single-cell-based models can be computationally prohibitive for large number of cells. For example, millions of cells were incubated in Armstrong's experiments in Figure 1. It is impractical to treat such large cell population sizes using single-cell-based models. Moreover, it is difficult to deal with single-

Figure 3: Time-lapse microscopy of a co-culture of HEK293 cells expressing nectin-1 and EGFP and those expressing both nectin-1 and mCherry. Time t indicates elapsed time in hours. Before the time-lapse experiments, these cells were separately cultured to allow the formation of independent colonies. When their colony edges came into contact with one another, their boundaries were examined. (a) Boundary between two colonies of HEK293 cells, which were doubly transfected with a nectin-1 and EGFP (green) or nectin-1 and mCherry (red). Cells expressing identical nectin types did not intermingle at the border. (b) Boundary between two colonies of HEK293 cells, which were doubly transfected with a nectin-1 and EGFP (green) or nectin-3 and mCherry (red). Cells expressing different nectin types intermingled at the border, and mutually invaded the counter colony.

cell-based models for realistic numbers of cells from analytical points of view. Thus, a continuous mathematical model is desirable. Armstrong, Painter and Sherratt (2006) have proposed a continuous model for cell-cell adhesion. They represented cell-cell adhesion as a nonlocal term. The model is attractive and challenging, so it is used and investigated by many researchers both from modelling and analytical points of view (e.g., Armstrong et al. (2009); Chaplain et al. (2011); Dyson et al. (2010); Painter et al. (2010)). However, biologically unrealistic behaviours are observed from numerical experiments. Especially, their model can not replicate Togashi et al. experiments shown in Figure 3 at all. In this paper, we identify the problem and remedy the model. Then we propose a modified continuous mathematical model for cell-cell adhesion. The modified model is able to replicate the Steinberg's and Togashi et al. experiments.

This paper is organized as follows. In the next section, we present Armstrong-Painter-Sherratt model. The derivation of the model is described briefly and numerical simulations are carried out to make out the problems of the model. We amend their model and propose a modified continuous model for cell-cell adhesion in Section 3. We demonstrate its ability to replicate different types of cell sorting phenomena. The paper concludes with discussions of the results.

2. Armstrong-Painter-Sherratt model

In this section, we introduce the model by Armstrong, Painter and Sherratt (2006) (APS model) and observe numerical simulations in order to understand the problems clearly and compare to our modified model.

2.1. Derivation

First, the model of a single population of cells is described. The population density at position x and time t is denoted by $u(x, t)$. We begin with the following conservation law:

$$\frac{\partial u}{\partial t} = -\nabla \cdot \mathbf{J},$$

where $\mathbf{J}$ is the flux of the cells. APS model is based on random walk and movement due to adhesion, that is, $\mathbf{J}$ consists of the fluxes due to diffusion $\mathbf{J}_d$ and adhesion $\mathbf{J}_a$. The diffusive flux follows Fick's law, namely,

$$\mathbf{J}_d = -c_d \nabla u.$$

Here c_d is the diffusion coefficient. The adhesive flux is proportional to the density and inversely proportional to cell size, namely:

$$\mathbf{J}_a = \frac{\phi}{R} u \mathbf{F},$$

where ϕ is a constant of proportionality related to viscosity, R is a positive constant called sensing radius, and $\mathbf{F}$ is the total force acting on the cells. The adhesion velocity vector $\mathbf{F}$ in d spatial dimension takes the form of a nonlocal term:

$$\begin{aligned} \mathbf{F}(\mathbf{x}) &= \mathbf{K}(u)(\mathbf{x}) \\ &= \int_0^R \int_{S^{d-1}} a g(u(\mathbf{x} + r\boldsymbol{\eta})) \omega(r) r^{d-1} \boldsymbol{\eta} d\boldsymbol{\eta} dr, \end{aligned}$$

where a is an adhesive strength parameter, S^{d-1} is the d -dimensional unit spherical surface, for $d = 1$ S^0 contains only the two points and the integral becomes a sum, the function g represents how the adhesive force depends on the local cell density, ω describes how the force is dependent on the direction and the distance from x . The

function g is given by

$$g(u) = \begin{cases} u(1 - u/m) & \text{if } u < m, \\ 0 & \text{otherwise,} \end{cases}$$

where m denotes the crowding capacity of the population. The function $g(u)$ increases monotonically in u at low cell density, and decreases for u above some threshold. The latter implies a resistance against moving into dense regions. To put all the pieces together, we obtain the following APS model for one population:

$$\frac{\partial u}{\partial t} = c_d \Delta u - \frac{\phi}{R} \nabla \cdot (u \mathbf{K}(u)).$$

In this work, we add the logistic growth term to APS model, that is,

$$\frac{\partial u}{\partial t} = c_d \Delta u - \frac{\phi}{R} \nabla \cdot (u \mathbf{K}(u)) + bu(1 - u/k).$$

Here b represents the growth rate and k is the carrying capacity. Putting

$$\begin{aligned} x^* &= \frac{x}{R}, & t^* &= \frac{c_d}{R^2} t, & u^* &= \frac{u}{m}, \\ a^* &= \frac{mR\phi}{c_d} a, & b^* &= \frac{b}{m}, & k^* &= \frac{k}{m}, \end{aligned} \quad (1)$$

and dropping the stars, we obtain the following non-dimensional APS model with a growth term:

$$\frac{\partial u}{\partial t} = \Delta u - \nabla \cdot (u \mathbf{K}(u)) + f(u), \quad (2)$$

where

$$\mathbf{K}(u)(\mathbf{x}) = \int_0^1 \int_{S^{d-1}} a g(u(\mathbf{x} + r\boldsymbol{\eta})) \omega(r) r^{d-1} \boldsymbol{\eta} d\boldsymbol{\eta} dr, \quad (3)$$

$$g(u) = \begin{cases} u(1 - u) & \text{if } u < 1, \\ 0 & \text{otherwise,} \end{cases} \quad (4)$$

$$f(u) = bu(1 - u/k). \quad (5)$$

The one population model (2) can be extended to a model of two populations. We note that each type of cells moves randomly and that cells of one type adhere to themselves (self-adhesion) and to cells of another cell type (cross-adhesion). APS model with growth terms of two population densities u and v is as follows:

$$\begin{cases} \frac{\partial u}{\partial t} = \Delta u - \nabla \cdot (u \mathbf{K}_1(u, v)) + f_1(u, v), \\ \frac{\partial v}{\partial t} = \Delta v - \nabla \cdot (v \mathbf{K}_2(u, v)) + f_2(u, v). \end{cases} \quad (6)$$

Here the functions $\mathbf{K}_1$ and $\mathbf{K}_2$ are given by

$$\begin{aligned} \mathbf{K}_1(u, v)(\mathbf{x}) &= \int_0^1 \int_{S^{d-1}} \left[a_{11} g_{11}(u(\mathbf{x} + r\boldsymbol{\eta}), v(\mathbf{x} + r\boldsymbol{\eta})) \right. \\ &\quad \left. + a_{12} g_{12}(u(\mathbf{x} + r\boldsymbol{\eta}), v(\mathbf{x} + r\boldsymbol{\eta})) \right] \omega(r) r^{d-1} \boldsymbol{\eta} d\boldsymbol{\eta} dr, \\ \mathbf{K}_2(u, v)(\mathbf{x}) &= \int_0^1 \int_{S^{d-1}} \left[a_{21} g_{21}(u(\mathbf{x} + r\boldsymbol{\eta}), v(\mathbf{x} + r\boldsymbol{\eta})) \right. \\ &\quad \left. + a_{22} g_{22}(u(\mathbf{x} + r\boldsymbol{\eta}), v(\mathbf{x} + r\boldsymbol{\eta})) \right] \omega(r) r^{d-1} \boldsymbol{\eta} d\boldsymbol{\eta} dr, \end{aligned} \quad (7)$$

where a_{ij} ($i, j = 1, 2$) denote (rescaled) adhesive strength parameters between the i th and j th cell populations, and the functions g_{ij} are defined by

$$\begin{aligned} g_{11}(u, v) &= g_{21}(u, v) \\ &= \begin{cases} u(1 - u - v) & \text{if } u + v < 1, \\ 0 & \text{otherwise,} \end{cases} \\ g_{22}(u, v) &= g_{12}(u, v) \\ &= \begin{cases} v(1 - u - v) & \text{if } u + v < 1, \\ 0 & \text{otherwise.} \end{cases} \end{aligned}$$

The growth terms f_1 and f_2 in (6) are defined by

$$\begin{aligned} f_1(u, v) &= b_1 u (1 - (u + v)/k_1), \\ f_2(u, v) &= b_2 v (1 - (u + v)/k_2), \end{aligned} \quad (8)$$

where b_i and k_i are the (rescaled) growth rate and carrying capacity of the i th population, respectively.

2.2. Numerical scheme

In this paper, we carry out numerical experiments in fixed domains $(-L, L)$ in one space dimension and $(-L, L) \times (-L, L)$ in two dimension. For the Steinberg experiments, we choose $L = 5$, the growth rates $b_i = 0$ (no growth terms) and impose periodic boundary conditions. We define the function $\omega \equiv 1$ in all simulations. We employ the standard explicit upwind finite volume method to discretize the problems (see Eymard, Gallouët and Herbin (2000)). We use uniform control volumes with sizes $h = 2L/N$ ($N = 1000$) in 1D and $h^2 = (2L/N)^2$ ($N = 160$) in 2D. The integral can be calculated exactly (except for numerical errors) in 1D since the finite volume solutions are constant in each control volume.

In 2D case, we use a numerical scheme to approximate the nonlocal terms. For each control volume, the adhesive velocities at four points $A-D$ in Figure 4 (a) are required. We explain how to calculate the approximation of the velocity vector $\mathbf{K}(u)(\mathbf{x})$ in (3) at the point A in Figure 4 (a). The unit circle with center $\mathbf{x}$ is divided into N_r regions uniformly in the radial direction. Here N_r is a user-prescribed positive integer. Each ring is discretized uniformly along the angular direction. Let N_θ be a (approximate) number

Figure 4: (a) A control volume which is a square h on a side and four points where the adhesive velocities have to be calculated. (b) The discretization of the unit circle with center A and with $N_r = 10$ and $N_\theta = 42$. The plots illustrate the barycenters of each grids. (c) The discretization of the unit circle with center B and with $N_r = 10$ and $N_\theta = 42$.

of grids of the outermost ring. The i th ring from the center is divided into $N_{\theta i} := \langle N_\theta i / N_r \rangle$ grids. Here we define $\langle s \rangle = 4 \max\{n/4 : n \in \mathbb{Z}, n \leq s\} + 2$ for $s \in \mathbb{R}$. We choose the barycenter of each grid as a representative point. The barycenter of ij grid ($i = 1, \dots, N_r$, $j = 1, \dots, N_{\theta i}$) is described by $\mathbf{x} + r_i \boldsymbol{\eta}_{ij}$, where r_i is the distance from $\mathbf{x}$ to the barycenter and $\boldsymbol{\eta}_{ij} = \left(\cos\left(\frac{2\pi j}{N_{\theta i}} + \frac{\pi}{2}\right), \sin\left(\frac{2\pi j}{N_{\theta i}} + \frac{\pi}{2}\right) \right)$. See Figure 4 (b) for the discretization of the circle. The area of the ij grid is denoted by S_i . Let U be a finite volume solution at time t . We calculate an approximation $\tilde{\mathbf{K}}(U)(\mathbf{x})$ of $\mathbf{K}(u)(\mathbf{x})$ as follows:

$$\tilde{\mathbf{K}}(U)(\mathbf{x}) = \sum_{i=1}^{N_r} r_i \omega(r_i) S_i \sum_{j=1}^{N_{\theta i}} g(U(\mathbf{x} + r_i \boldsymbol{\eta}_{ij})) \boldsymbol{\eta}_{ij}.$$

We introduced a notation $\langle \cdot \rangle$ and chose barycenters as representative points so that each representative point does not overlap with edges of the control volumes. The approximation of the velocity vector at the point B in Figure 4 (a) is similarly calculated but $\boldsymbol{\eta}_{ij}$ is replaced by $\boldsymbol{\eta}_{ij} = \left(\cos\left(\frac{2\pi j}{N_{\theta i}}\right), \sin\left(\frac{2\pi j}{N_{\theta i}}\right) \right)$ (see Figure 4 (c)). We choose $N_r = 10$ and $N_\theta = 42$ in all numerical experiments in this paper. We compute the numerical integration directly, and it requires high computational cost. It is very useful to employ fast Fourier transform techniques in order to save computational time. As for the technique, refer Gerisch (2010).

2.3. Visualization

In Figures of 1D simulations, we use green line to plot u and red line to plot v , and total population density $u + v$ is drawn with a blue line behind green and red lines. Appearance of blue line implies that the two types of cells are mixed. Under the green (resp. red) line is painted in translucent green (resp. pink) so that we can easily see whether the two populations are mixed or not.

In 2D simulations, we use translucent green (resp. translucent red) to plot u (resp. v). The region where $u, v < 10^{-3}$ is painted in white.

2.4. Numerical simulations for the Steinberg experiments

We carry out numerical simulations for the Steinberg's cell sorting experiments shown in Figure 2 using APS model (6) without the growth terms. Figure 5 shows the numerical results in 1D and Figure 6 illustrates the results in 2D. We note that cross-sections of the 2D numerical solutions resemble the 1D solutions. Armstrong, Painter and Sherratt (2006) concluded that they can demonstrate excellent agreement between APS model and the cell sorting experiments. That was because they drew plane views colored by the ratio of u and v , and cut off at some level (probably. Details of visualization were not written in their paper). However, looking at 1D simulations shown in Figure 5 and bird's eye view of 2D simulations shown in Figure 6, it is difficult to accept their conclusion. In the cell sorting experiments, we can clearly see the boundary of the tissues. However, it is difficult to detect the boundary from the solution. Engulfment pattern in Figure 6 (b) is different from density distribution imagined from Figure 1. Steinberg (1962a,b,c) noted that properties of sorting-out cell systems are similar to those of two-phase systems of mutually immiscible liquids such as oil and water. In contrast, the two types of cells are mixed (we can see blue lines in Figure 5), even in complete sorting case (d). We can observe other unrealistic phenomena from numerical simulations. For example, cell clusters of one type of cells separated by a cell colony of another type of cells fuse across the cell colony. Another example is that a cell cluster is getting bigger, and another cluster is getting smaller and disappear even though there is no cell birth or death in the system. It is difficult to say that APS model is able to replicate behaviours of cell populations.

2.5. Numerical simulations for the Togashi et al. experiments

We perform numerical simulations for the Togashi et al. experiments shown in Figure 3. We carry out simulations in 1D since the phenomena appear nearly 1D. The following actual measured values are used:

$$\begin{aligned} c_d &= 9.87 \text{ } \mu\text{m/h}, \\ R &= 100 \text{ } \mu\text{m}, \\ b_1 &= b_2 = 1/12 \text{ times/h}, \\ k_1 &= k_2 = 0.0748 \text{ cells}/\mu\text{m}. \end{aligned}$$

The Sensing radius R is determined by the length from the cell body to the tip of leading edge. The distance between the initial colonies is 500 μm . The self-adhesive strength of nectin-1 expressing cells is 30RU (Response Unit), that of nectin-3 expressing cells is 8RU, and the cross-adhesive strength between those cells is 230RU. As for these adhesive strengths, see Harrison et al. (2012). We could not measure the values ϕ and m_i , so we choose $\phi = 1$ and $m_i = k_i$. These are not measured values but these may not have much influence on the behaviours of the numerical solutions. We consider the domain of length

Figure 5: Numerical results of APS model (6) for the Steinberg experiments in one space dimension. Initial data and numerical solutions at times $t = 0.2, 0.5, 1, 2, 10$ are shown. Figures (a)–(d) illustrate the results using different adhesive strength parameters: (a) Mixing, $a_{11} = 60$, $a_{12} = a_{21} = 60$, $a_{22} = 60$. (b) Engulfment, $a_{11} = 120$, $a_{12} = a_{21} = 30$, $a_{22} = 15$. (c) Partial engulfment, $a_{11} = 60$, $a_{12} = a_{21} = 30$, $a_{22} = 60$. (d) Complete sorting, $a_{11} = 60$, $a_{12} = a_{21} = 0$, $a_{22} = 30$.

2400 μm , that is, the computational domain is $(-L, L)$ with $L = 12$. The following Dirichlet boundary conditions are imposed:

$$\begin{aligned} u(x, t) &= 0, \\ v(x, t) &= k_2/m_2 = 1 \quad \text{if } x \leq -L, t > 0, \\ u(x, t) &= k_1/m_1 = 1, \\ v(x, t) &= 0 \quad \text{if } x \geq L, t > 0. \end{aligned}$$

The numerical results are shown in Figure 7. The numerical experiments (a) and (b) correspond to the experiments shown in Figure 3 (a) and (b), respectively. The time t indicates elapsed time in hours on the original time scale. The colony edges do not contact with one another at similar time to the experiments although the simulations are based on empirical data. The oscillations near the edges are due to the choice of $\omega \equiv 1$. More realistic form may be a smooth function such that $\omega(1) = 0$.

We carry out numerical experiments using a large diffusion coefficient $c_d = 200$. This diffusion coefficient is chosen so that the two colonies merge around $t = 30\text{h}$. The actual measured values are used for the other parameters. Figure 7 (c) and (d) illustrate the numerical results with the large diffusion coefficient corresponding to Figure 3 (a) and (b), respectively. The two populations are mutually invading the counter colonies after contact in both cases. It is difficult to find the differences between the two behaviours in (c) and (d). The numerical solutions behave in the same way despite attempts at various adhesive strength parameters and diffusion coefficients. The two populations intermingle with one another even if there is

no cross-adhesion. APS model can not replicate the phenomena in Togashi et al. experiments at all.

3. A modified model

We would like to develop a continuous model which replicates fundamental behaviours associated with cell-cell adhesion. Let us think back on the derivation of APS model (2). They assume random movement of each cell. However, it is unlikely that cells move randomly from experimental observations. Cells are not able to move randomly because they adhere to each other in the first place. Precisely speaking, they seem to be moving randomly, but the random movement is very small compared to the other dynamics. Looking at Togashi et al. experiments (Figure 3), the cells seem to be moving towards lower density area. Therefore, we change underlying idea of cell movement from “cells move randomly” to “cells move from high to low pressure regions”. Figure 8 shows trajectories of cells which helps the reader to understand above observations.

3.1. One population model

We start from the conservation of mass:

$$\frac{\partial u}{\partial t} = -\nabla \cdot (u\mathbf{V}),$$

where the velocity vector $\mathbf{V}$ is composed of velocities due to pressure $\mathbf{V}_p$ and adhesion $\mathbf{V}_a$. We assume that the pressure p is proportional to the population density, namely,

$$\mathbf{V}_p = -\nabla p = -c_p \nabla u.$$

Figure 6: Numerical results of APS model (6) for the Steinberg experiments in two space dimension. Randomly mixed initial data and numerical solutions at times $t = 0.1, 1, 10, 50$ are shown. The mixture ratio of cells is 50 : 50. Figures (a)–(d) illustrate the results using different adhesive strength parameters: (a) Mixing, $a_{11} = 60$, $a_{12} = a_{21} = 60$, $a_{22} = 60$. (b) Engulfment, $a_{11} = 120$, $a_{12} = a_{21} = 30$, $a_{22} = 15$. (c) Partial engulfment, $a_{11} = 60$, $a_{12} = a_{21} = 30$, $a_{22} = 60$. (d) Complete sorting, $a_{11} = 60$, $a_{12} = a_{21} = 0$, $a_{22} = 30$.

Figure 7: Numerical results of APS model (6) for the Togashi et al. experiments shown in Figure 3. Initial data and numerical solutions at times $t = 24, 48, 72, 96, 200$ hours on the original time scale are shown. Figures (a) and (b) illustrate the results based on empirical data, and figures (c) and (d) show the results with a large diffusion coefficient. The adhesive strength parameters are $a_{11} = a_{12} = a_{21} = a_{22} = 30$ in (a) and (c) which correspond to the experiments in Figure 3 (a), and $a_{11} = 30, a_{12} = a_{21} = 230, a_{22} = 8$ in (b) and (d) which correspond to the experiments in Figure 3 (b).

Here c_p is called the dispersivity. We use the same adhesion velocity vector as in APS model, that is,

$$\mathbf{V}_a = \frac{\phi}{R} \mathbf{K}(u).$$

Adding the logistic growth term, we obtain the following model:

$$\frac{\partial u}{\partial t} = c_p \nabla \cdot (u \nabla u) - \frac{\phi}{R} \nabla \cdot (u \mathbf{K}(u)) + bu(1 - u/k).$$

We rescale the problem in a similar fashion to (1) where c_d is replaced with c_p , and drop the stars to obtain the following non-dimensional model:

$$\frac{\partial u}{\partial t} = \nabla \cdot (u \nabla u) - \nabla \cdot (u \mathbf{K}(u)) + f(u). \quad (9)$$

Here, the functions $\mathbf{K}$ and f are defined in (3)–(5).

The difference between (2) and (9) is that one follows linear diffusion and the other satisfies nonlinear diffusion of porous medium type. The diffusion in (9) vanishes at the points where $u = 0$. The vanishing diffusion characterizes the presence of a free boundary between regions where $u > 0$ and where $u = 0$.

Now let us look at numerical simulations of (2) (Figure 9 (a)) and of (9) (Figure 9 (b)) without growth terms. We choose the adhesive strength parameter $a = 60$. In either case, it can be seen that colonies are being formed. The colonies formed from (2) are with very broad bases because of the linear diffusion, and the bases of the colonies formed from (9) are tight due to the degeneracy of diffusion. In (2) the cells will aggregate a single colony in the end because each colony interacts with far distance colonies. In (9) the

Figure 8: Tracking of the trajectories of the cells corresponding to Figure 3. X in the left figures indicate the selected cells. Time t indicates elapsed time in hours. Each red cell moves from the left to the right and each green cell moves from the right to the left before contact. The random movement has a small impact on their behaviour.

Figure 9: (a) A numerical result of APS model (2). (b) A numerical result of the modified model (9). Initial data and numerical solutions at times $t = 0.1, 1, 10$ are shown.

numerical solution at time $t = 10$ in Figure 9 (a) is close to a steady state since there is no interaction between distant colonies. Thus the behaviours of solutions of (2) and (9) are completely different from mathematical points of view. However, there might not be a dime's worth of difference between the two equations from a biological point of view. We will realize that their difference is essential in consideration of two or more cell populations.

3.2. Two populations model

We take into account two cell populations u and v . The mass conservation yields

$$\frac{\partial u}{\partial t} = -\nabla \cdot (u \mathbf{V}^u), \quad \frac{\partial v}{\partial t} = -\nabla \cdot (v \mathbf{V}^v).$$

Each of the velocity vectors $\mathbf{V}^u$ and $\mathbf{V}^v$ consists of two types of velocities, i.e.,

$$\mathbf{V}^u = \mathbf{V}_p^u + \mathbf{V}_a^u, \quad \mathbf{V}^v = \mathbf{V}_p^v + \mathbf{V}_a^v.$$

Assuming that the pressures p^u and p^v are proportional to the population density of total cells which is now $u + v$, we have

$$\mathbf{V}_p^u = -\nabla p^u = -\nabla(u + v), \quad \mathbf{V}_p^v = -\nabla p^v = -\nabla(u + v).$$

Here the constant of proportion is rescaled to be one. Therefore each type of cells disperses locally toward lower values of total population. The same adhesion velocity vectors (7) and growth terms (8) as in APS model are employed:

$$\mathbf{V}_a^u = \mathbf{K}_1(u, v), \quad \mathbf{V}_a^v = \mathbf{K}_2(u, v).$$

Thus we obtained the following system as a possible model for cell-cell adhesion of two populations:

$$\begin{cases} \frac{\partial u}{\partial t} = \nabla \cdot (u (\nabla(u + v) - \mathbf{K}_1(u, v))) + f_1(u, v), \\ \frac{\partial v}{\partial t} = \nabla \cdot (v (\nabla(u + v) - \mathbf{K}_2(u, v))) + f_2(u, v). \end{cases} \quad (10)$$

The fundamental movement is no longer diffusion. The system consists of the first term on the right-hand side of

(10), that is,

$$\begin{cases} \frac{\partial u}{\partial t} = \nabla \cdot (u (\nabla(u + v))), \\ \frac{\partial v}{\partial t} = \nabla \cdot (v (\nabla(u + v))) \end{cases} \quad (11)$$

was derived by Gurtin and Pipkin (1984) using the mass conservation law and the assumption that the dispersal velocity is proportional to the gradient of the total population density (Thus our idea is the same as theirs). The system (11) with or without growth terms have been studied from mathematical points of view (e.g., Bertsch et al. (1985, 1987, 2010, 2012, 2013)). The most striking feature of the system (11) is that the system admits completely segregated solutions, i.e., $uv = 0$. If initial data are completely segregated then there exist solutions which are segregated for all time. Such solutions u, v have jump discontinuities at the segregation front.

3.3. Numerical simulations for the Steinberg experiments

Numerical calculations are performed for the Steinberg experiments using (10) without growth terms. Results in 1D are shown in Figure 10. Except for the mixing state (a), we can clearly see that the numerical solutions u, v are completely segregated (blue lines do not appear at $t = 10$), and u and v are discontinuous at the segregation fronts. Figure 11 shows numerical results in 2D. Also in 2D simulations, except for (a), we can see that the numerical solutions are completely segregated because they are drawn by transparent colors. Engulfment pattern in Figure 11 (b) coincides with density distribution imagined from Figure 1. Single central masses of green cells are surrounded by red cells. Intermediate stages of cell sorting are also replicated. Comparing Figure 1 (b) with Figure 11 (b) at $t = 10$, multiple internal green cell clusters embedded in red tissue. We can capture each of the four types of pattern formation seen in the cell sorting experiments outlined in Figure 2.

3.4. Numerical simulations for the Togashi et al. experiments

Togashi et al. experiments are simulated by the modified formulation (10). The actual measured values stated in Section 2.5 are used. We choose $c_p = 35$. This dispersivity is determined from the speed of the colony edges since existence of a traveling wave solution to (9) is expected and the speed of the traveling wave will be determined by c_p from a mathematical point of view.

Numerical results are shown in Figure 12. We observe travelling wave like behaviours in early stages of cultures, and their colony edges come into contact with one another. In case where strengths of self- and cross-adhesion are equal (a) which corresponds to the experiment shown in Figure 3 (a), they do not overlap at later times. After filling the region, they will stop growing and moving.

Figure 10: Numerical results of the model (10) for the Steinberg experiments in one space dimension. Initial data and numerical solutions at times $t = 0.2, 0.5, 1, 2, 10$ are shown. Figures (a)–(d) illustrate the results using different adhesive strength parameters: (a) Mixing, $a_{11} = 60$, $a_{12} = a_{21} = 60$, $a_{22} = 60$. (b) Engulfment, $a_{11} = 120$, $a_{12} = a_{21} = 30$, $a_{22} = 15$. (c) Partial engulfment, $a_{11} = 60$, $a_{12} = a_{21} = 30$, $a_{22} = 60$. (d) Complete sorting, $a_{11} = 60$, $a_{12} = a_{21} = 0$, $a_{22} = 30$.

Figure 12: Numerical results of the model (10) for the Togashi et al. experiments. The simulations are based on empirical data. Initial data and numerical solutions at times $t = 24, 48, 72, 96, 200$ hours on the original time scale are shown. The adhesive strength parameters are $a_{11} = a_{12} = a_{21} = a_{22} = 30$ in (a) which correspond to the experiments in Figure 3 (a), and $a_{11} = 30$, $a_{12} = a_{21} = 230$, $a_{22} = 8$ in (b) which correspond to the experiments in Figure 3 (b).

Thus the modified model (10) is able to successfully capture the contact inhibition of growth and movement. In the numerical experiment (b) which corresponds to the experiment shown in Figure 3 (b), we can observe that they are slowly intermingling with one another after contact. We conclude that the modified model (10) can replicate the different types of phenomena observed in cell-cell adhesion experiments.

4. Discussion

In this paper, we have considered continuum models for cell-cell adhesion. We have studied the model by Armstrong, Painter and Sherratt (2006) (APS model). We observed numerical solutions of APS model for the Steinberg experiments and the Togashi et al. experiments. The two types of cells are mixed in the numerical results although the experiments show that the two cell populations are mutually immiscible. For the Togashi et al. experiments, even though we carried out the numerical simulations using the actual measured values, the numerical solutions behaved unrealistically. APS model was unable to replicate the cell sorting patterns observed experimentally. We observed unrealistic phenomena from numerical simulations. The underlying cause of the problem was that APS model is based on random walk. From experimental observations, in this paper, we changed underlying idea of cell movement from “cells move randomly” to “cells move from high to low pressure regions”. Then we proposed a modified continuous model for cell-cell adhesion. The modified model is able to replicate not only the different types of sorting

Figure 11: Numerical results of the model (10) for the Steinberg experiments in two space dimension. Randomly mixed initial data and numerical solutions at times $t = 0.1, 1, 10, 50$ are shown. The mixture ratio of cells is 50 : 50. Figures (a)–(d) illustrate the results using different adhesive strength parameters: (a) Mixing, $a_{11} = 60$, $a_{12} = a_{21} = 60$, $a_{22} = 60$. (b) Engulfment, $a_{11} = 120$, $a_{12} = a_{21} = 30$, $a_{22} = 15$. (c) Partial engulfment, $a_{11} = 60$, $a_{12} = a_{21} = 30$, $a_{22} = 60$. (d) Complete sorting, $a_{11} = 60$, $a_{12} = a_{21} = 0$, $a_{22} = 30$.

patterns but also the phenomenon of contact inhibition of growth and movement.

Armstrong, Painter and Sherratt (2006) mentioned nonlinear diffusion in their paper. They wrote “an appropriate form of diffusive flux may be non-linear and decreasing with cell density.” However, as long as diffusion is adopted, the two cell populations will always intermingle with each other. Therefore the Steinberg experiments and the Togashi et al. experiments can not be replicated. The modified model did not start from random movements and is able to replicate the immiscible phenomena. The modified model (9) for a single population is also regarded as a nonlinear diffusion problem from a mathematical point of view. But it is just the contrary to what they think. In a case where we regard the modified problem as diffusion, the diffusive flux equals to the cell density, that is, the flux increases with cell density. When we consider two populations model, we realize clearly that the fundamental movement in the modified model is no longer diffusion.

In this paper, we removed the diffusion terms since the impact of random movement is very small compared to the other dynamics and we were considering the essence of cell behaviours. However, precisely speaking, they are moving randomly. Therefore, more realistic model may be as follows:

$$\begin{cases} \frac{\partial u}{\partial t} = \varepsilon \Delta u + \nabla \cdot (u(\nabla(u+v) - \mathbf{K}_1(u,v))) + f_1(u,v), \\ \frac{\partial v}{\partial t} = \varepsilon \Delta v + \nabla \cdot (v(\nabla(u+v) - \mathbf{K}_2(u,v))) + f_2(u,v), \end{cases}$$

where the diffusion coefficient ε is very small as written in Section 2.5. This formulation also has an advantage from a mathematical viewpoint. Adding diffusion terms often facilitate its analysis.

Armstrong, Painter and Sherratt introduced a nonlocal advection term in order to represent cell-cell adhesion. In this paper, we pointed out the importance of rethinking about the bottom of a problem. A number of models related to cell dynamics might employ linear diffusion for the sake of simplicity. However, while we consider behaviours of dense cell populations such as cell-cell adhesion, it is difficult to think of random movement of each cell. It might be nice to get back to basics and make fundamental reforms if solutions give unrealistic behaviours.

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