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Role of Differential Adhesion in Cell Cluster Evolution: from Vasculogenesis to Cancer Metastasis

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Abstract

Cell-cell and cell-matrix adhesions are fundamental to numerous physiological processes, including angiogenesis, tumorigenesis, metastatic spreading, and wound healing. We employ cellular potts model to computationally predict the organization of cells within a 3D matrix. The energy potentials regulating cell-cell (J_{CC}) and cell-matrix (J_{MC}) adhesive interactions are systematically varied to represent different, biologically relevant adhesive conditions. Chemotactically induced cell migration is also addressed. Starting from a cluster of cells, variations in relative cell adhesion alone lead to different cellular patterns such as spreading of metastatic tumors and angiogenesis. The combination of low cell-cell adhesion (high J_{CC}) and high heterotypic adhesion (low J_{MC}) favors the fragmentation of the original cluster into multiple, smaller cell-clusters (metastasis). Conversely, cellular systems exhibiting high homotypic affinity (low J_{CC}) preserve their original configuration, avoiding fragmentation (organogenesis). For intermediate values of J_{CC} and J_{MC} (i.e. $J_{CC}/J_{MC} \sim 1$), tubular and corrugated structures form. Fully developed vascular trees are assembled only in systems where contact-inhibited-chemotaxis is activated upon cell contact. Also, the rate of secretion, diffusion and sequestration of chemotactic factors, cell deformability and motility; do not significantly affect these trends. Further developments of this computational model will predict the efficacy of therapeutic interventions to modulate the diseased microenvironment by directly altering cell cohesion.

Keywords

Cellular organization; Pattern formation; Cell-cell adhesion; Cell-matrix adhesion; Chemotaxis; and Cellular potts model

Introduction

Biological tissues are composed of a multitude of cells, with different functions, tridimensionally organized within an extracellular matrix (ECM). This is an intricate network of fibrous proteins arranged around the cells that provides structural support, a molecular depot with nutrients and growth factors, and transfer and integration of mechano-

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chemical stimuli for cell proliferation and migration [1]. The spatial arrangement of cells is fundamental to the process of tissue morphogenesis [2], wound healing [3], development of pathological conditions, such as cancer and atherosclerotic plaques [4, 5], and in shaping tissue specific biological functions. The temporal evolution and rearrangement of biological tissues is rooted in the way the individual resident cells interact with their microenvironment via adhesion molecules, and with the secretion and adsorption of soluble factors.

Cell adhesion is known to play a critical role in the multiscale organization of both normal and pathological tissues [6]. A large number of different transmembrane proteins, typically identified as cell adhesion molecules (CAMs), regulate the homophilic (cell-cell adhesion) and heterophilic binding (cell-matrix adhesion) of cells. Typically, cadherin molecules mediate cell-cell adhesion, whereas integrins connect the cells with ECM. E-cadherin is the most relevant adhesion molecule in the formation of epithelial tissues [7–9]. In vasculogenesis and angiogenesis, the organization of endothelial cells is modulated by adhesive interactions with the ECM through β_1 integrins and with other endothelial cells through VE-cadherins [10, 11]. In tumor spreading and metastasis, the so-called epithelial-to-mesenchymal transition (EMT) is associated with a reduction in E-cadherin expression and functionality, therefore favoring the tumor cell interaction with ECM and consequent migration [2, 3, 8]. As such, predicting the adhesion dynamics of cells and their spatial reorganization in biological tissues may have implications on the cure and prevention of diseases.

Computational models for understanding and predicting cellular morphogenesis fall within three major categories: continuum, discrete and hybrid models [12–22]. In continuum models, the dependent variables, such as the cell population density, the secretion, diffusion and degradation of growth factors, nutrients and chemicals are modeled using a set of ordinary and partial differential equations, imposing mass and momentum balance [12]. Discrete models, such as those based upon cellular automata or cellular potts model, use a set of deterministic and/or probabilistic rules to describe the cell behavior [23]. These models can easily incorporate biological signals defining cell-cell and cell-matrix interactions involving chemotaxis, haptotaxis and durotaxis. Finally, hybrid approaches integrate continuum and discrete models, where individual cells dynamically evolve in response to continuous changes in the governing parameters.

In this paper, we use the cellular potts model (CPM) to study the organization of cells within a three-dimensional lattice emulating ECM. The model considers one cell type and deals with cell-cell and cell-matrix adhesive interactions. The importance of such interactions in morphing the original cell cluster is systematically analyzed over a wide range of biologically relevant conditions, including contact-inhibition of chemotactic signals. A sensitivity analysis is also performed to elucidate the importance of cell population density, chemotaxis and cell motility as compared to adhesion. The tridimensional compactness C of the cell cluster is computed for all different configurations of the system as a function of adhesion, chemotaxis and cell motility.

Material and Methods

2.1 Computational model for the 3D organization of the cell

The cellular potts model (CPM) [13, 14], -a hybrid cellular automata-partial differential equation model- is used here to analyze the spatial organization of cells in ECM. The CPM represents Glazier-Graner-Hogeweg (GGH) formalism implemented in the CompuCell3D open source software [13–15]-is a lattice-based stochastic model which uses the principle of energy minimization to compute the equilibrium configuration at a lower energy state. CPM model uses a set of sites $\vec{l}$ on a lattice to describe a biological cell $\sigma(\vec{l})$. The present model contains one type of cell immersed in a matrix (ECM), treated as a large, generalized cell. Unless specified, the cell-lattice dimensions are set to $100 \times 100 \times 100$ with each individual cell occupying a $4 \times 4 \times 4$ lattice cube in the initial configuration. One lattice site has a linear size of $4 \mu m$; thus the size of the simulation domain corresponds to $400 \mu m$ and one simulated cell is $16 \mu m$, approximately the size of an epithelial cell [16].

Molecular binding and adhesion is specified at the intra (cell-cell) and inter (cell-matrix) cell interfaces. J_{CC} is the potential energy associated with cell-cell adhesion, and J_{MC} is the potential energy related to the cell-matrix adhesion. Cells reorganize to favor stronger rather than weaker cell-cell and cell-matrix adhesions [10], i.e. an increase in cell-cell (-matrix) adhesion is responsible for a reduction in J_{CC} (J_{MC}). For simplicity, it is assumed that cells have fixed surface properties (i.e. surface density of adhesion molecules) and their overall volumes are almost constant while the cells can still deform, i.e. elongate and contract. We assume that cells do not divide or grow within the short time of the considered evolution (~320 hrs). The cadherin and integrin families of adhesion molecules mediate the hemophilic and heterophilic cell binding.

The Hamiltonian for the system can be written as [13, 14]

(1)

which includes three terms: i) the variation in adhesive energy between two neighboring cells, and between cells and extracellular matrix (ECM); ii) the variation in energy associated with the deformation of the cell; iii) the variation in the cell's coupling to an external field, i.e. chemotaxis where external chemical gradients guide cell movement in the direction of higher or lower chemical concentration [10, 17]. The third-nearest neighbor lattice sites are used for calculating the energy resulting from cell-cell adhesion, which reduces lattice anisotropy effects compared to first-neighbor calculations [18].

The indices $\vec{l}$ and $\vec{j}$ identify neighboring lattice sites; τ denotes cell type; J is the adhesive energy per unit area which is symmetric $J(\tau, \tau') = J(\tau', \tau)$; $v(\sigma)$ represents the actual volume in the lattice sites of a cell, $\sigma(\vec{l})$, which is constrained to a target volume, V_b ; λ_{vol} measures the cells resistance to compression; c is the concentration of the chemical substance, assumed present everywhere in a layer of extracellular matrix under cells, and μ is the

chemotaxis coefficient. Chemotaxis is assumed to depend linearly on the concentration of a chemical substance.

The time evolution of the system is obtained by *Monte Carlo* simulations with the Metropolis algorithm. First, the cell index of a randomly chosen source voxel is substituted with that of a neighboring target voxel as a trial. Next, the change in the Hamiltonian between before and after the trial, ΔH , is calculated. The probability of accepting a new configuration due to such a change is [19]

(2)

where, T represents cell membrane fluctuations in the units of energy which defines the intrinsic cell motility due to thermal fluctuations. One *Monte Carlo Step (MCS)* corresponds to n attempts, where n is the total number of cell lattice sites [19]. In the CPM model, each lattice cell i moves according to the change in the Hamiltonian due to chemical gradient; thus velocity at each lattice site is equal to $\mu \nabla c$, where c is the local chemical concentration [20, 21].

2.2 Autocrine Signaling and Chemotaxis

The chemoattractant molecules are self-consistently generated by the cells, i.e., autocrine signaling. It is assumed that cells uniformly secrete a diffusible chemical substance at rate α , and that the chemical spreads through the ECM with diffusion constant D . Due to proteolytic enzymes or binding to ECM components, the chemical also decays at rate ε . Thus, the spatial concentration c of the autocrine signaling obeys the reaction-diffusion equation [10, 22, 23]

(3)

where M denotes matrix cells, $\delta\{\alpha(\vec{r}), M\} = 1$ in the ECM and 0 inside endothelial cells. To implement contact inhibited chemotaxis, we set $\mu = 0$ at cell-cell boundary interface in eqn (1). Here, contact inhibited chemotaxis ensures that cell-cell interfaces do not chemotax; however cell-matrix boundary interfaces chemotax towards matrix cells [10, 19].

2.3 3D morphometrics

Geometry reconstruction is the first step in determining the 3D cellular morphology. We characterize the cell-cluster morphologies in terms of numerically measured morphometric by calculating the *compactness* C of the cell clusters. Compactness is the fraction of solid material inside the convex hull of the 3D shape, also known as shape factor, $C = V_{object} / V_{hull}$, where V_{object} is the volume of the cells in a cluster, and V_{hull} is the volume of its convex hull [24]. Convex hull is the smallest convex set containing the cluster, or it is a rubber membrane wrapped around the entire cluster. Thus, $C = 1$ represents a sphere, while $C = 0$ represents fragmented (or dispersed) morphology [24]. Geometry of the cell-cluster is reconstructed using tetrahedral finite element method in open source package, TetGen [25]

(Figure 1c). We generate tetrahedral mesh for the volume and convex hull of the cell-clusters, where generated tetrahedrons are defined by the three polyhedron edge vectors $\vec{a}$, $\vec{b}$, and $\vec{c}$ from a given polyhedron vertex. Next, the volume of a tetrahedron is calculated using $V = (1/6)|\vec{a} \cdot (\vec{b} \times \vec{c})|$ [26]. Finally, the total volume and the volume of the convex hull, are obtained by summing the volume of tetrahedrons [27].

2.4 Average square displacement (ASD) and cell velocity

To characterize cell motility, we calculate the average squared displacement for all the cells in the cluster [28]. It is defined as $\overline{\Delta r^2} = \frac{1}{N} \sum_{i=1}^N \Delta r_i^2$ where

$\Delta \vec{r}_i$ is the vector displacement of a cell in time t . Instantaneous velocity of the cell i at time t is calculated using, $\vec{v}_i = \frac{\Delta \vec{r}_i}{\Delta t}$.

2.5 Parameter values

(Table 1) We set the target cell volume in eqn (1) to $V_t = 74 \mu m^3$, $\lambda_{vol} = 20$ and $\mu = 5000$ for all cells, which defines cellular properties [16]. Unless we specify otherwise, we set $T = 5$, where T represents the cell membrane fluctuation amplitude in the units of energy. We set the chemical secretion rate by cells $\alpha = 0.22 \times 10^{-3}/sec$ [10, 12], its decay rate $\varepsilon = 0.27 \times 10^{-3}/sec$ [10, 12] and its diffusion constant $D = 0.042 \mu m^2/sec$ [22, 29–32].

Results

3.1 Simulations of the cellular morphogenesis

The initial tridimensional configuration is a cluster of 62-cells (Figure 1a). Using the CPM model, the cell reorganization is studied as a function of the relative adhesion. The system evolves towards different configurations as schematically drawn in Figure 1, forming aggregates (cluster configuration), tubular and branched structures (tubular configuration), and fragmented patterns (dispersed configuration). The cell-cluster morphology can be quantified using the compactness C , defined as the ratio between the total volume of the cells forming the cluster ($V_{cluster}$) and the volume of the corresponding convex hull (V_{hull}) where $C = 1$ for a sphere and $C = 0$ for a perfectly fragmented morphology [24].

To investigate the role of cell-cell and cell-matrix adhesion in the cellular pattern formation, simulations were run by varying adhesion parameters while keeping all the other parameters fixed. The compactness for each simulated configuration is plotted as a function of the relative response of cell-cell and cell-matrix adhesion, J_{CC}/J_{MC} . Note that J_{CC} and J_{MC} are two independent parameters. Their ratio, J_{CC}/J_{MC} , is a measure of the relative importance of cell-cell adhesion over cell-matrix adhesion in a specific configuration of the system. This relative adhesion is known experimentally to affect the temporal evolution and rearrangement of cells in their microenvironments [2, 3, 6–8]. Therefore, J_{CC} and J_{CC}/J_{MC} were chosen as the two independent parameters for predicting the temporal evolution of the cell-clusters. Note that higher J_{CC} (or J_{MC}) implies that cell-cell (or cell-matrix) adhesion, is less energetically favorable. (Figure 2) For a given cell-cell cohesion, J_{CC} , compactness decreases from the initial aggregated cluster ($C = 1$), to a tubular cluster (known as intermediate state), and finally to a dispersed cluster ($C = 0$), as a function of cell-matrix

adhesion, J_{MC} . For smaller relative adhesion, J_{CC}/J_{MC} , cells tend to adhere together; and for larger relative adhesion, tend to separate and migrate through surrounding ECM, thus forming a dispersed morphology. Thus, the relative adhesion regulates the tendency of the cells to either disperse or adhere together.

For large values of J_{CC} (> 10), the compactness curves exhibit a local maximum for J_{CC}/J_{MC} ranging between 1 and 3, denoting an increase in surface corrugation of the cell cluster. Small protrusions can be clearly seen to develop out of smooth surface. This is an intermediate state preluding to an extensive fragmentation of the cluster that inevitably occurs for larger ratios J_{CC}/J_{MC} .

Figure 3 summarizes the morphologies of 3D clusters as a function of cell-cell (J_{CC}) and cell-matrix (J_{MC}) adhesions. After 20,000 Monte Carlo Steps (~ 320 hrs of real time), initiated from a 62-cell cluster, the initial cluster reorganizes to attain an aggregated, tubular (or branched), and dispersed morphologies as a function of cell-cell and cell-matrix adhesions. Compactness increases with increasing cell-cell cohesion and decreases with increasing relative cell-matrix adhesion.

3.2 Contact-inhibited chemotaxis

In contact-inhibited chemotaxis, at the interface between two adjacent cells, chemotactic stimuli are inactivated ($\mu = 0$). With the loss of cell-cell contact-inhibited chemotaxis, the initial cluster organizes to attain only aggregated and dispersed morphologies. Tubular (or branched) morphology does not appear under this condition. Figure 4a shows change in compactness with/without contact-inhibited mechanism for J_{CC} equal 10 and 20. The change in the compactness is much slower compared to the contact-inhibited case where initial aggregated cell-clusters rearrange themselves into a dispersed cluster as a function of relative adhesion. Note that in non-contact inhibited cases, compactness decreases monotonically over the relative adhesion J_{CC}/J_{MC} .

To distinguish between contact inhibited and non-contact-inhibited cases, we compare the trajectories of cells at equivalent cell-cell and cell-matrix adhesive energies ($J_{CC} = J_{MC}$ at equivalent surface tension). Figure 4b shows representative trajectories of a randomly selected single cell, from a 62-cell cluster, in contact-inhibited and non-contact inhibited cases. In the first case, the cell appears less constrained in its motion within the cluster, as compared with the non-contact inhibited case. This is also documented by the average cell displacement (Figure 4c) and cell velocity (Figure 4d), which are larger for the cells with contact-inhibited chemotaxis. This was expected as in clusters where contact inhibits chemotaxis and the consequent cell motility, cells can move more freely, being less constrained by chemotactic stimuli. The observed behavior is in agreement with experimental data on endothelial cells [33].

3.3 Sensitivity Analysis

In order to understand which parameters of the model are significant in determining the final configurations of cell-clusters, sensitivity analysis was performed on parameters. The model was described using five reference parameters. The compactness of the cell-cluster was compared by varying the parameter values from 50 to 200%. Each run was initiated with a

62-cells cluster and simulated for 20,000 MCS (~ 320 hrs) by keeping other parameters fixed.

3.3.1 Effect of chemotaxis and secretion rate—Figure 5a, shows that the secretion rate, α , affects the final configuration of cell-cluster; since cells move randomly up or down the chemical gradient, reduction in the secretion rate, results in slower decay of compactness i.e., slower fragmentation, while increment of the secretion rate results in faster decay of compactness i.e., faster fragmentation. Perturbation in the chemotaxis coefficient μ has a linear effect in the cellular pattern formation (Figure 5b), which was expected from eqn.4. Stronger chemotaxis produces faster loss of compactness, mainly due to increased cell velocity. Cell velocity in the GGH model satisfies (eqn.1), $u \approx \mu \nabla \vec{c}$, where c is the local chemical concentration [20, 21]. The perturbation in the cell's resistance to compression, λ , does not affect the final configuration of cell-clusters (Figure 5c), although it shows small change of compactness, less than 0.5%. Figure 5d shows the plot of average compactness as function of time. Error bars show standard deviations over 4 simulations.

3.3.2 Effect of the cell membrane fluctuation amplitude (T)—To investigate influence of the cell membrane fluctuation amplitude on cellular pattern formation, we ran five simulations shown in, with fluctuation amplitude set to $T = 0.1, 5, 10, 20$ and 100 (Figure. 6). To study the effect of cell membrane fluctuation amplitudes, cell-cell and cell-matrix adhesion forces are kept equivalent. Low T produces immotile cells whereas high values of T gives rise to extremely motile cells with ragged cell boundaries. Low and high values of T represent extreme cases and outside “normal” T ranges used in a well-tuned GGH model [14].

3.3.3 Effect of Cell density—As we vary the initial cell density from a 62 to a 130 and 257 cells cluster, the final configuration of the system and the evolution patterns do not change (Supplementary Figures SF1 and SF2), implying that the initial cell density is not a relevant parameter in this specific study.

Discussion

Modulation of homotypic (cell-cell) and heterotypic (cell-matrix) adhesion is a fundamental step in the development of organs, in the spatial organization of biological tissues and in the development of diseases [34–38]. Adhesion molecules belonging to the cadherin and integrin families are the major biological switchers playing an important role in modulating cell differential adhesion. The cadherin molecules exhibit organ dependent distribution patterns that suggest their dominant role in the spatial organization of cells during the developmental process [39–41]. Targeting these molecules with antibodies inhibiting their adhesive functions has led to significant alterations in the developmental processes and tissue organization [42]. On the other hand, integrins are expressed ubiquitously within the ECM of any tissue and mediate heterotypic adhesive interactions with collagen, fibrinogen, fibronectin and vitronectin forming the ECM fibrous component [43]. These molecules are responsible for cell-ECM communication and affect cell apoptosis, differentiation, survival and migration. For this reason, integrins too play a relevant role in organogenesis, tissue development and disease progression [43, 44].

In the progression of solid malignancies, cells within the primary tumor mass undergo a complex transformation, known as the epithelial-to-mesenchymal transition (EMT), where homotypic cell cohesion is progressively substituted by cell-matrix and heterotypic cell adhesion [35, 37, 38]. One of the earliest steps in this process is the down regulation and loss of expression of adhesion molecules, such as E-Cadherin, and the corresponding upregulation of N-Cadherin, a molecule that is generally associated with an increased migratory propensity of the cells. Indeed, N-Cadherin is believed to facilitate the heterotypic adhesive interaction of tumor cells with fibroblasts, populating the tumor stromal compartment, and endothelial cells. In other words, EMT modulates the relative adhesion of the cells decreasing the cohesion among the tumor cells and increasing their capacity to interact with other, different cell families and the extracellular matrix [2, 4, 5]. This has been shown to be a necessary condition for the disruption of the original individual tumor mass and the subsequent dispersion of the tumor cells into the surrounding tissue. Based on the presented analysis, EMT can be described by an increase in J_{CC} , in that the down regulation of E-Cadherin increases the energy threshold for activating homotypic cell-cell adhesive interaction; and a decrease in J_{MC} , in that the up-regulation of N-Cadherin and other adhesion molecules reduces the energy threshold for activating heterotypic cell-cell and cell-matrix adhesive interaction. Therefore, referring to Figure 2, a solid, individual tumor is characterized by a high compactness C , tending in the limit to unity, which is associated with low energy ratio J_{CC}/J_{MC} and a low J_{CC} . At the other extreme, a metastasis mass is more properly described by a fragmented configuration, with low C , originating from the original compact cellular system as a consequence of an increase in J_{CC} and J_{CC}/J_{MC} , i.e. a reduction in J_{MC} . Based on this, it can be speculated that silencing N-Cadherin or other molecules that are associated with the migration of cells, such as the Aquaporins [45], could reverse the metastatic process by simply increasing J_{MC} . Future developments of the presented computational model should offer a tool for predicting the response of a cellular system with different compactness C ; i.e. at different stages of development, to silencing therapies.

Intermediate between the formation of a homogeneous system of cells and the fragmentation of a system of cells is the case of vasculogenesis which derives by the proper balance between homotypic adhesion of adjacent endothelial cells and heterotypic adhesion of the endothelial cells with the surrounding matrix [46–49]. This interplay leads to the moderate values of J_{CC} and J_{MC} which disfavor either system fragmentation or system clustering, hence intermediate compactness C , is typical of tubular structures. Vascular endothelial cadherin (VE-cadherin) and the platelet-endothelial cell adhesion molecule-1 (PECAM-1) are considered to be involved in homotypic endothelial cell adhesion and in the formation of inter-endothelial junctions, which are important for lumen formation, cell polarity, and vascular permeability [49]. These molecules are also responsible of balancing the J_{CC} value during vasculogenesis. Specific antibodies to these integrins, as well as small molecule mimetics, have been shown to inhibit tumor-induced angiogenesis [45, 50]. Recent experimental evidence suggests that the substrate stiffness can also play a role in directing the formation of vascular networks [50, 51]. Rigid gel substrates have been shown to promote the formation of stable focal adhesion spots and the spreading of cells thus favoring the heterotypic adhesive interaction between the cell and the substrate. Differently, on more

deformable substrates cells tend to migrate together to form larger clusters thus reducing their interaction with the surrounding environment. In other words, compliant substrate would increase J_{MC} supporting the formation of cellular structures with larger compactness C ; in contrast, rigid substrates would limit cell-cell homotypic interactions by increasing the energy threshold J_{CC} . Note that contact inhibition of chemotaxis is critical in supporting the formation of tubular structure as demonstrated by Figure 4, in agreement with the literature [10, 19].

In summary, the cellular Potts model has been used to systematically analyze the effect of differential cell adhesion of the spatio-temporal organization of cellular clusters. It has been shown that the balance between homotypic and heterotypic cell adhesion regulates cell patterning, whereas chemotactic signaling can only alter the rate of cluster reorganization but not its final configuration. In agreement with the epithelial-to-mesenchymal transition (EMT) observed in tumor metastatization, poor homotypic adhesion (high J_{CC}) and strong heterotypic affinity (low J_{MC}) favors the fragmentation of the original blob in multiple, individual smaller cell clusters. Differently, a fine balance between cell-cell and cell-matrix adhesion ($J_{CC}/J_{MC} \sim 1$) leads to corrugated cellular clusters, with small fingers, and to tubular structures resembling vascular trees in the case of contact-inhibited chemotaxis. This computational model will help in predicting the outcome of therapeutic interventions aimed at modulating the diseased microenvironment by directly altering cell cohesion.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Refereces

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Figure 1. Schematic of distinct 3D cell-organization

(a) Initial configuration of a cluster (blob) of cells which is made up of 60 generalized cells, represents a spherical morphology with 2D and 3D view, (b) represents clustered, tubular, corrugated, fragmented morphologies, (c) first two panels represent the volume of 3D cluster, whereas the third panel represents the convex hull of the 3D cluster. Geometry reconstruction is performed using tetrahedral mesh via TetGen open source package [25], and then volume of the cluster, and volume of the convex hull of the cluster is calculated (see Material and Methods).

Figure 2. Plot of compactness versus relative adhesion

Plot of compactness of a 62-cell cluster after 20,000 Monte Carlo Step (~ 320 hrs) as a function of the relative adhesion (J_{CC}/J_{MC}) for different cell-cell adhesion (J_{CC}), where $J_{CC} = 40$ represents lower level of adhesion than $J_{CC} = 1$. The compactness $C = 1$ represents a spherical morphology, where $C = 0$ represents fragmented (or dispersed) morphology [24]. Dotted circle represents increase in compactness where morphologies changes from vascular to corrugated structure.

Figure 3. 3D simulated morphologies as a function of cell-cell and cell-matrix adhesions (J_{CC} , J_{MC} , J_{CC}/J_{MC}), where J_{CC} describes cell-cell adhesion, J_{MC} represents cell-matrix i.e., cell-matrix adhesion, and J_{CC}/J_{MC} represents relative adhesion. Plot of minimum energy morphologies after 20,000 Monte Carlo Step (~ 320 hrs) that was initiated from a 62-cell cluster as a function of the relative adhesion, J_{CC}/J_{MC} , where C represents the compactness that is a measure of shape, also known as shape factor. We define compactness as a fraction of solid material inside the convex hull of a shape.

Figure 4. Effect of contact-inhibited mechanism

(a) Tubular morphology is missing in the non-contact-inhibited case: Dashed line – Non-contact-inhibited-chemotaxis, Solid line –Contact-inhibited-chemotaxis; (b) For 62-cell cluster, cell trajectory of a single cell in a contact-inhibited-chemotaxis and in a non-contact inhibited-case, at 10 min interval from 0 to 80 hrs, where cell-cell and cell-matrix adhesive energies are kept equal ($J_{CC} = J_{MC}$, at equivalent surface tension); (c) Average squared displacement of a 62-cell cluster from initial positions over time for 10 simulated experiments, in contact-inhibited (solid curve) and non-contact-inhibited (dashed curve) cases; (d) Average cell velocity of a 62-cell cluster during 4 hrs interval over 10 simulated experiments for contact-inhibited (solid curve) and non-contact-inhibited (dashed curve) cases.

Figure 5. Sensitivity analysis of the parameters in the proposed model

(a) Secretion rate α , (b) Chemotaxis coefficient μ , (c) the cells resistance to compression λ , while keeping the other parameters constant, (d) Average compactness C of 3D simulation as a function of time. Error bars show standard deviations over 4 simulations.

Figure 6. Effect cell membrane fluctuation amplitude
At $T=0.1, 10, 20$ and 100 .

Table 1

Parameters used in the computational model.

Symbol	Physical Meaning	Value	Source
1 MCS	Time step	0.017 hr	
1 voxel	Spatial dimension	4 μm	
1 cell	Simulated size	16 μm	[16]
α	Secretion Rate	$0.22 \times 10^{-3}/sec$	[10, 12]
ε	Decay rate	$0.27 \times 10^{-3}/sec$	[10, 12]
D	Diffusion constant	0.042 $\mu m^2/sec$	[22, 29–32]
V_t	Maximum cell volume	74 μm^3	[15, 16]
λ_{vol}	Cell resistance to compression	20	[15, 16]
μ	Chemotaxis coefficient	5000	[15, 16]
T	Cell membrane fluctuation amplitude	5	[15, 16]