



Universiteit
Leiden
The Netherlands

Modelling of concentration inhibited and autocrine driven branching

Roest, B. van der

Citation

Roest, B. van der. (2014). *Modelling of concentration inhibited and autocrine driven branching*.

Version: Not Applicable (or Unknown)

License: [License to inclusion and publication of a Bachelor or Master thesis in the Leiden University Student Repository](#)

Downloaded from: <https://hdl.handle.net/1887/3596524>

Note: To cite this publication please use the final published version (if applicable).

Modelling of concentration inhibited and autocrine
driven branching

Bastiaan van der Roest

December 18, 2014

Supervisor: Roeland Merks

Contents

1	Introduction	3
2	Material and Methods	7
2.1	Biological explanation	7
2.2	Cellular Potts Model	7
2.3	Chemotaxis and inhibition	8
2.3.1	Inhibition Model	9
2.3.2	Epithelial Mesenchymal Model	9
2.4	Computational input	10
2.5	Morphospaces and compactness	11
3	Results	13
3.1	Inhibition Model	13
3.1.1	Diffusion length	15
3.1.2	Temperature	19
3.1.3	Inhibition constant	23
3.1.4	Conclusion	28
3.2	EC-Mesenchym model	28
3.2.1	Diffusion length	31
3.2.2	Temperature	38
3.2.3	Chemotaxis constant	42
3.2.4	Conclusion	46
4	Discussion	47
5	References	49
6	Appendix	50
6.1	XML files of IM and EMM	50
6.2	Steppables	54

1 Introduction

The development of many different organs, such as the lungs, kidneys and all kind of glands, is driven by the branching of epithelial tissue. This particular kind of morphogenesis is responsible for the tree-like forms of the organs, by sprouting of new tubes from preexisting ones (Affolter et al, 2003). Branching morphogenesis is regulated by growth factors, extracellular matrix (ECM) molecules, proteases and morphogens. A complex system with all of these cues together tells a tissue to branch or not (Nelson, 2006). A question that arises is whether epithelial tissue alone can form a branching morphology, or does it need external signals to initiate branching? Does the epithelial cells has a intrinsic power to branch or does it need other organ tissue such as the mesechymal tissue?

C.M. Nelson et al. (2006) studied how the geometry of a tissue determines branching morphogenesis. In this article they found that the geometry is determined by autocrine inhibitory morphogens. These are substances, produced by the epithelial cells, that inhibits the neighboring cells to move into the direction of the substances. Figure 1 shows one of the results of the experiments done by Nelson.

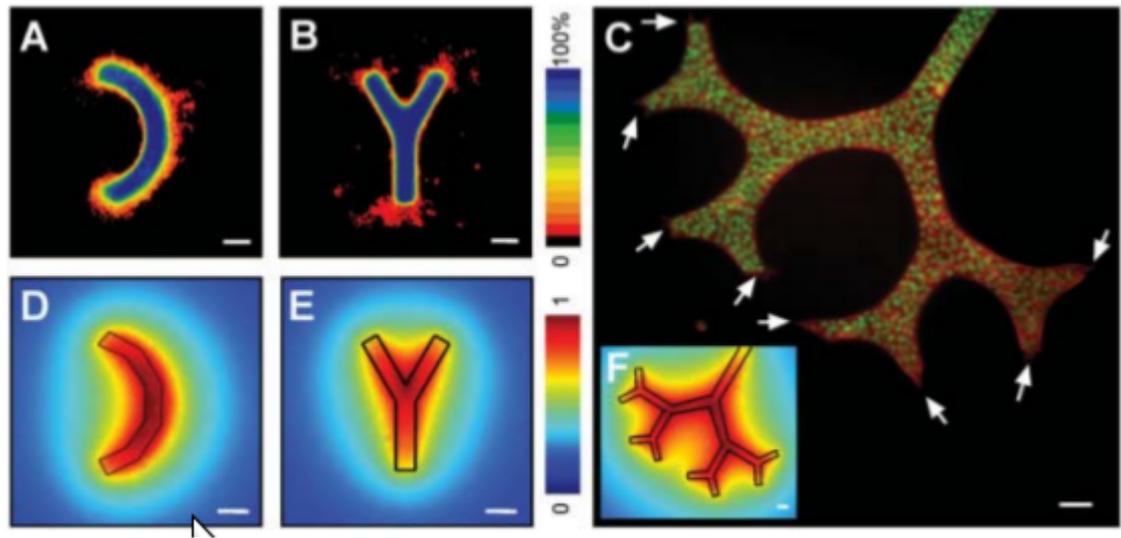


Figure 1: Branching position is determined by tubule geometry and is consistent with the concentration profile of secreted diffusible inhibitor(s). Scale bars, 50 μm

In Figure 1 frequency maps - stacked fluorescent images of stained nuclei from 50 tubules to quantify the position of cells - of 24 hours after induction of branching are shown for (A) curved tubules, (B) bifurcated tubules and (C) a tree. The occurrence of branching sites in (C) are found by the arrows. Images (D), (E) and (F) show concentration profiles of the inhibitors. In this case for (D) curved tubules, (E) bifurcated tubules and (F) fractal trees. The concentration profiles show that growth occurs at the branching sites. In the frequency maps these places are the ones with the least concentration of inhibitor. So branching occurs at that places where the cells are at least inhibit to move. In Figure 2 the cells are placed near each other to see how the cells react.

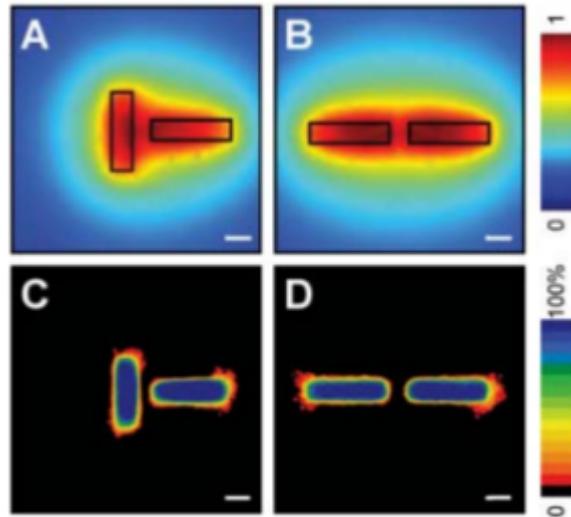


Figure 2: Position of branching can be predicted by calculated concentration profiles. Scale bars, 50 μm

Images (A) and (B) in Figure 2 are concentration profiles of the inhibitors and show that the most concentration of inhibitor is placed right between the cells. The frequency maps (C) and (D) 24 hours after induction of branching conform this idea that the cells are inhibit to move in the region with high concentration of inhibitor.

In this article it will be discussed if it is possible to model the experiments of Nelson et al., to see if epithelial tissue can form branching morphologies by the autoinhibition described in the experiments.

An other article, by T. Hirashima et al (2009), shows a model of the epithelial cells inside mesenchymal tissue. They modelled the uterine bud inside

a kidney and placed two sources of growth factor GDNF inside the mesenchymal tissue. Besides placing sources of growth factor they also let the epithelial tissue be elastic, such that the modeled uteric bud does not fall apart. By placing the sources of GDNF and setting the tissue to be elastic, they found that the uteric bud form branches. The initial conditions are given in Figure 3.

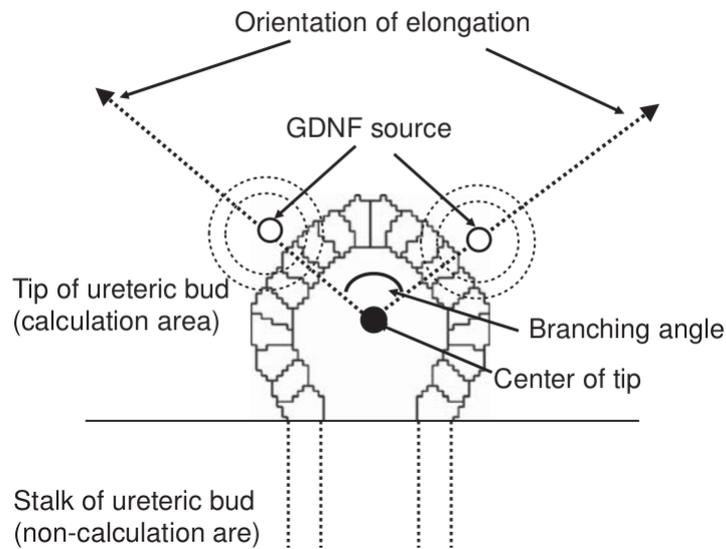


Figure 3: Computer simulation by Hirashima et al. (2009)

Figure 3 shows a tip of an ureteric bud with dashed lines the stalk part that is not affected by the GDNF. The black circle is the center of the tip and the other circles represents the GDNF sources. Results of computer simulations of the model described by Hirashima are given in Figure 4.

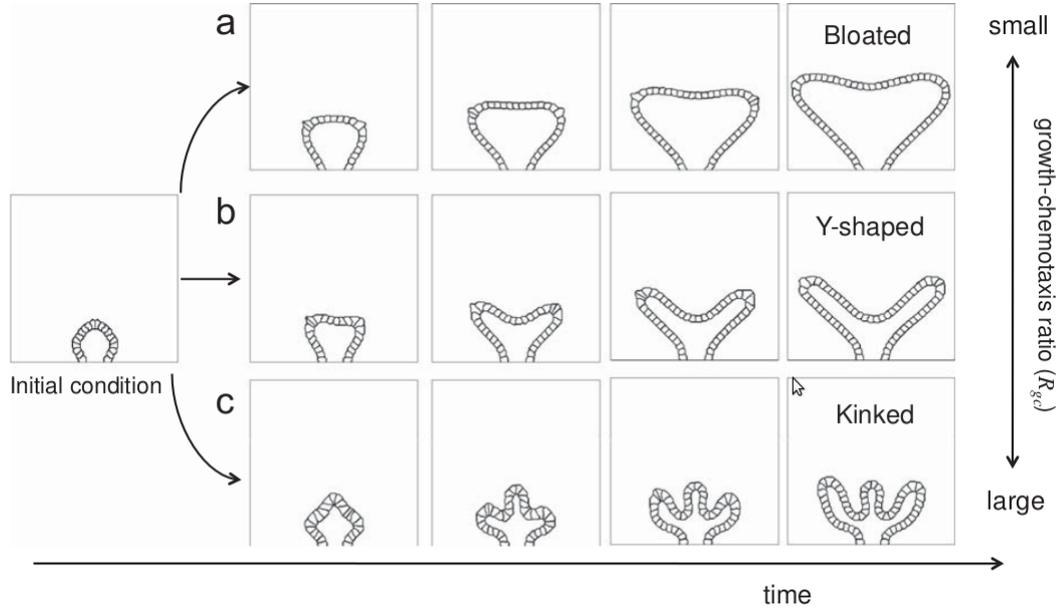


Figure 4: Temporal change in the shape of a ureteric bud

The results in Figure 4 show that a proper value of the growth-chemotaxis ratio (R_{gc}) is necessary. This ratio is defined as the growth rate of a layer divided by the velocity of cell movement due to chemotaxis. The growth-chemotaxis ratio should be in balance to form a Y-shaped structure (b). When the growth rate is higher, the initial pattern will form into a kinked pattern (c) and when the cell mobility is too high a bloated structure is formed.

To make this model a slightly more realistic, this article will discuss an expansion of their model, by introducing an autocrine loop between the epithelial and mesenchymal cells. In this way, the model itself chooses where the sources of the growth factor will be inside the mesenchymal tissue. Then it is more realistic to see whether branching morphologies occur in the epithelial tissue due to a reaction with the mesenchymal tissue or not, because in nature the sources of the growth factor are not set at particular places in the mesenchymal tissue but are randomly spread among the mesenchymal tissue. This randomly spread is given by the expansion of the model by Hirashima et al. Besides the random placement of growth factor sources will the epithelial tissue not be elastic. In nature cells can behave both individually as together at a different way, so they do not have to be stitched together in an elastic way.

2 Material and Methods

2.1 Biological explanation

As said before, this article describes experiments with two computational models. The first one, further named as the Inhibition Model (IM), models the autoinhibition of the epithelial cells following the physical experiments of Nelson *e.a.* (2006). A cell secretes a substance, for example the growth factors EGF or TGF β , that inhibits itself and its neighbors to move into the direction with the higher concentration of the substance. So a cluster of cells will form branches into directions with the lowest local concentration of inhibitor, because the cells are inhibited to walk into other directions.

The other model, named as the Epithelial-Mesenchymal Model (EMM), computes the interaction between the epithelial cells and the mesenchymal tissue. The model includes an autocrine loop between the two kinds of cells. That means that the epithelial cells secrete a substance c_1 that activates the mesenchymal cells to secrete a substance c_2 . This c_2 is a chemoattractant for the epithelial cells, so when the mesenchymal cells are activated to secrete c_2 , the epithelial cells will move through the mesenchymal tissue. However, this movement is not equivalent on all sides on the epithelial cluster. Because of the instability of the cluster, it can occur that at some sites more mesenchymal cells are activated by c_1 , so the chemotaxis to c_2 is stronger at these sites. In this way, the epithelial cluster can form branches within the mesenchymal tissue.

2.2 Cellular Potts Model

Both models can be computed by an expansion of the Cellular Potts Model (CPM), also called the Glazier-Graner-Hogeweg (GGH) Model. Within this model the cells are represented as a set of sites or pixels on a lattice. Each site \vec{x} is part of a specific cell, indicated as $\sigma(\vec{x})$. The properties of this cell differ depending on which type of cell it is. Therefore a site also gets an indication for the cell type: $\tau(\sigma(\vec{x}))$. To model the motility of cells, the lattice sites have to be able to change, in such a way that one site can copy itself into another. In nature everything wants to be in the state that costs the least energy, so for a realistic model the cells in the model also wants to be in the state of the least energy. To measure the energy over the lattice, the CPM model has the following Hamiltonian:

$$H = \sum_{\text{neighbors}} J(\tau(\sigma(\vec{x})), \tau(\sigma(\vec{x}')))(1 - \delta(\sigma(\vec{x}), \sigma(\vec{x}'))) + \lambda \sum_{\sigma} (a(\sigma) - A_{\text{target}}(\sigma))^2$$

Here the $J(\tau(\sigma(\vec{x})), \tau(\sigma(\vec{x}')))$ describes the energy of the bond between sites \vec{x} and \vec{x}' , depending on the cell types of the sites. The Kronecker delta gives

$$\delta(\sigma(\vec{x}), \sigma(\vec{x}')) = \begin{cases} 1 & \text{if } \sigma(\vec{x}) = \sigma(\vec{x}') \\ 0 & \text{if } \sigma(\vec{x}) \neq \sigma(\vec{x}') \end{cases}$$

so that the energy is zero if the sites are from the same cell. Only the bonds between different cells count inside the Hamiltonian.

The parameter λ stands for the resistance to compression of the cells. Cells also have an area $a(\sigma)$ and a fixed target area $A_{\text{target}}(\sigma)$.

The Hamiltonian gives the effective energy of the lattice by measuring two components: the total adhesion energy, that is the energy of all the bonds between different cells, and the surface component. That last one gives that the cell area should be conserved. It can happen that the model lowers the current area beneath the target area, so that the surface component become positive, but then the adhesion energy can decrease a little bit more to gain a lower effective energy.

The model will minimize the effective energy of the lattice. Physically this means that the cells will go to their lowest energy state and thus that cells only leave their lowest energy state by adding force. When cells move to the lowest energy state they thus exerts an active force on the environment. To minimize the effective energy, the model has to make copies of lattice sites into others by a stochastic process. Cells move randomly up or down, left or right, so a lattice site choose randomly a neighbor to copy itself in. When the copy is associated with an increase in the pattern energy, that is as the effective energy will decrease after the copy is done, the probability that this copy will actually be done is 1. With a copy associated with a decrease in the pattern energy, the probability is $e^{-\frac{\Delta H}{T}}$ where ΔH is the change in effective energy and T is the cellular temperature or cell motility. So the probability that a copy succeeds, depending on the effective energy change, is given by

$$P(\Delta H) = \begin{cases} e^{-\frac{\Delta H}{T}} & \text{if } \Delta H \geq 0 \\ 1 & \text{if } \Delta H < 0 \end{cases}$$

The effective energy will be measured at each time step, when a site tries to copy itself into another.

2.3 Chemotaxis and inhibition

Both the IM and EMM are extensions of the Cellular Potts Model. The basic movements of the cells are computed by the CPM, but in both models the effective energy has an extra component.

2.3.1 Inhibition Model

First the Inhibition Model. The substance secreted by the epithelial cells inhibits the cells to move in the directions with high concentrations of the substance. So the effective energy change has to rise when a lattice site wants to copy itself into a site with a higher concentration inhibitor. Therefore, the effective energy change by the inhibition can be measured as

$$\Delta H_{\text{inhibition}} = \mu c(\vec{x}')$$

with μ the inhibition constant and $c(\vec{x}')$ the concentration inhibitor on lattice site \vec{x}' . This is a neighbor of \vec{x} , the site which want to copy itself in \vec{x}' . Because of the higher positive energy change, when copying into a lattice site with a higher concentration inhibitor, the probability $e^{\frac{-\Delta H}{T}}$ becomes lower, so the copy is less likely to succeed. And therefore the cell will better move into a direction with a lower concentration of inhibitor. So the morphogen inhibits cell movement.

2.3.2 Epithelial Mesenchymal Model

In the Epithelial Mesenchymal Model, instead of inhibiting the movements of cells in particular directions, the mesenchymal tissue attracts the epithelial cells. In terms of the effective energy it is more profitable to move into a direction with a higher concentration of chemoattractant, c_2 in the model. So the model has to check whether the concentration in the targeting site is higher or lower than in the copying site. The effective energy change can be given by¹

$$\Delta H_{\text{chemotaxis}} = -\mu \left(\frac{c(\vec{x}')}{1 + sc(\vec{x}')} - \frac{c(\vec{x})}{1 + sc(\vec{x})} \right)$$

with μ the chemotaxis constant, $c(\vec{x})$ the concentration in \vec{x} , $c(\vec{x}')$ the concentration in \vec{x}' and s the saturation constant, which will be zero in all the simulations in this article. When \vec{x}' contains a higher concentration chemoattractant than \vec{x} , the ΔH will be negative. So the probability that this copy will succeed will be 1. A lattice site will thus always copy itself into a neighbor, when the concentration c_2 of the neighbor is higher than in the site itself. In that way epithelial cells will move through the mesenchymal tissue driven by the concentration of c_2 .

The concentration of chemoattractant or inhibitor is given by the following formula:

$$\frac{\partial c}{\partial t} = \alpha(1 - \delta(\sigma(\vec{x}), 0)) - \varepsilon\delta(\sigma(\vec{x}), 0)c + D\nabla^2 c$$

¹Merks, R., Newmand S., Glazier, J. (2004) Cell-oriented modeling of in vitro capillary development. In: Soot P., Chopard, B., Hoekstra, A., eds. Cellular Automata: 6th International Conference on Cellular Automata for Research and Industry. Berlin: Springer Verlag, Lect. Notes Comput. Sc. 3305, 425-434

where $\delta(\sigma(\vec{x}), 0) = 0$ inside the secreting cells and $\delta(\sigma(\vec{x}), 0) = 1$ in the others cell types or medium. The constant α gives the secretion rate, ε the rate of degradation and D the rate of diffusion²³.

The effective range of the chemoattractant is given by the diffusion length L . These parameters can be derived from the differential equation for the concentration. Consider a steady state for the concentration, then $\frac{\partial c}{\partial t} = 0$, and take then the homogen differential equation

$$D\nabla^2 c - \varepsilon c = 0$$

General solutions for this equation are $c(\vec{x}) = c_0 e^{\lambda \vec{x}}$ where $\lambda = \sqrt{\frac{\varepsilon}{D}}$ or $\lambda = -\sqrt{\frac{\varepsilon}{D}}$. When $\lambda = \sqrt{\frac{\varepsilon}{D}}$ then the concentration will rise to infinity, so this solution is not possible, thus $c(\vec{x}) = c_0 e^{-\sqrt{\frac{\varepsilon}{D}} \vec{x}}$. The diffusion length is then defined as the length where the concentration is decreased with a factor e. So

$$c_0 e^{-\sqrt{\frac{\varepsilon}{D}} \vec{x}} = c_0 e^{-1}$$

thus⁴ $\vec{x} = \sqrt{\frac{D}{\varepsilon}} = L$.

2.4 Computational input

In this article the software CompuCell3D⁵ is used for simulating the two models with the computer. This program works with Python and XML files that describe the input of various subprograms. To measure the chemotaxis, CompuCell3D has a python file named Chemotaxis, that works out the partial differential equation of the concentration c_1 and c_2 and the change in effective energy for chemotaxis.

To rewrite the Chemotaxis file for simulating autoinhibition we rewrite

```
float ChemotaxisPlugin::simpleChemotaxisFormula(float _flipNeighborConc,
        float _conc, ChemotaxisData & _chemotaxisData){
return (_flipNeighborConc - _conc) * _chemotaxisData.lambda
}
```

into

²Gamba, A., Ambrosi, D., Coniglio, A., De Candia, A., Di Talia, S., et al. (2003) Percolation, morphogenesis, and Burgers dynamics in blood vessel formation. Phys Rev Lett 90

³Merks, R., Perryn, E., Shirinifard, A., Glazier J. (2008) Contact-Inhibited Chemotaxis in De Novo and Sprouting Blood-Vessel Growth. PLoS Comput Biol 4(9)

⁴Ambrosi, D., Gamba, A., Serini, G. (2004) Cell directional persistence and chemotaxis in vascular morphogenesis. B Math Biol 66: 1851-1873

⁵M. Swat, Gilberto L. Thomas, Julio M. Belmonte, A. Shirinifard, D.Hmeljak, J. A. Glazier (2012) Multi-Scale Modeling of Tissues Using CompuCell3D, Computational Methods in Cell Biology, Methods in Cell Biology 110: 325-366

```

float ChemotaxisPlugin::simpleChemotaxisFormula(float _flipNeighborConc,
        float _conc, ChemotaxisData & _chemotaxisData){
return (_flipNeighborConc)*_chemotaxisData.lambda;
}

```

The autocrine loop inside the EMM is made by writing the following code in the steppable for the model.

```

def step(self, mcs):
    field1=CompuCell.getConcentrationField(self.simulator,"c1")
    #veld van c1
    field2=CompuCell.getConcentrationField(self.simulator,"c2")
    #veld van c2
    for cell in self.cellListByType(2):
        x=int(cell.xCOM)           #x-waarde van cel
        y=int(cell.yCOM)           #y-waarde van cel
        field1_cell=field1[x,y,0]  #conc c1 bij cel
        field2_cell=field2[x,y,0]  #conc c2 bij cel
        if field1_cell >= 1:       #als conc c1 >= 1 dan
            field2_cell=field2_cell+0.2*field1_cell
            #verhoog conc c2 met helft conc c1
            field2[x,y,0]=field2_cell

```

This code finds for all mesenchymal cells the concentration c_1 and let the concentration c_2 rise with 0.2 times the concentration c_1 as the c_1 concentration is 1 at a mesenchymal cell. The source of extra c_2 is at the center of mass of the cell. So in the simulations dots can occur when looking at the c_2 field.

The XML file of the model exists of so-called plugins and steppables. Plugins give the initial conditions of the lattice site and steppables compute the changes at the lattice per one Monte Carlo Step. That is the time in which \mathcal{N} copy attempts are made, with \mathcal{N} the number of lattice sites. The following plugins and steppables are used for both models: Celltype, Volume, CenterOfMass, Contact, Chemotaxis, Secretion, FlexibleDiffusionSolverFE and PIFInitializer.

2.5 Morphospaces and compactness

During the simulations parameters sweeps are made. That means that the program automatically changes the value of a specific parameter during the simulations. The data from these simulations can then be used for analysis of the different parameter values. In this article two kinds of analysis will be done: making morphospaces and computing the compactness.

Morphospaces are images with sets of morphologies after a particular amount of Monte Carlo Steps formed with different values of one specific parameter. This article describes morphospaces for the temperature, the diffusion constant and the inhibition or chemotaxis constant, depending on which model is described.

Each parameter sweep contains 10 repeats of a simulation for each parameter. For each repeat the compactness of the epithelial tissue can be measured. The compactness is defined as the relation between the surface of a cluster of cells and its convex hull. The convex hull of a cluster of cells is the smallest convex set that contains this cluster. So the compactness is $C = \frac{A_{\text{area}}}{A_{\text{convex hull}}}$. The compactness can be seen as a measure for the spread of the cells through the plane and can tell if there are branches or not. A low compactness means that the surface of the convex hull is much bigger than the surface of the cluster, so that the cluster can contain branches. The repeats of the simulations give the possibility to calculate the standard deviation of the compactness to get an accurate value for the compactness. These standard deviations are always given after 10 simulations and are notated as the error of the compactness⁶.

⁶Palm, M., Merks, R. Large-scale parameter studies of cell-based models of tissue morphogenesis using CompuCell3D or VirtualLeaf

3 Results

Both the experiments of Nelson (2006) and the simulations done by Hirashima (2009) showed branched structures of epithelial cells. To test if Nelson’s observations suffice for branching growth, a model is made to simulate the movements of epithelial cells and the reaction on each other by autoinhibition. To make the simulations of Hirashima more realistic a model is made to simulate movements of epithelial cells inside mesenchymal tissue forming a autocrine loop.

3.1 Inhibition Model

Autoinhibition is the inhibition of a substance c_1 on the epithelial cells produced by the same cells. A cell produce c_1 that inhibits its neighbors to move into that direction. In that way, when one cell is slightly more outside the cluster, it inhibits its neighbors to move outside and itself can move further, so a branch occurs.

The model is first simulated with some chosen parameters, to see if branches occur. Next the model is computed with a parameter sweep of the temperature, the diffusionconstant and the inhibitionconstant. In that way, the sensitivity of the model to the parameters can be found.

First of all the inhibition model is computed with given values of the parameters. These values are chosen to give a good example of the model. They are given in table 1:

Table 1: Parameter values in computation of Inhibition Model

Parameter	Cell type	Value
Temperature		150
Adhesion energy	Medium - EC	15
	EC - EC	15
Chemotaxis constant		150
Secretion constant		0.013
Diffusion constant		0.24
Decay constant		0.03

The adhesion energy between medium and medium is always set to zero. Looking at the energy component of the Hamiltonian:

$$\sum_{neighbors} J(\tau(\sigma(\vec{x})), \tau(\sigma(\vec{x}')))(1 - \delta(\sigma(\vec{x}), \sigma(\vec{x}')))$$

when the adhesion energy is zero, this component is zero at every boundary between pixels with cell type medium because all the medium is of the same

σ . So the adhesion energy between pixels of type medium does not effect the energy component.

To see the effect of autoinhibition on a cluster of cells there is chosen for a rectangular cluster, such as in the experiments of Nelson. The initial cluster is given in Figure 5.

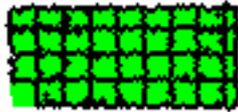


Figure 5: **Initial cluster of epithelial cells;** Cellfield after 0 MCS

Figure 5 shows a group cells in green, with in black the boundaries between the different cells. With the particular parameter values given in Table 1 the results are given in Figure 6.



Figure 6: **Inhibition by epithelial cells;** Cellfield after 9000 MCS

The cells move from their start position, whereas the concentration of inhibitor slightly change along the cluster. Some cells move out the cluster, causing an inhibition at the neighboring cells. This moving outwards of some cells, inhibiting others to move, causes the branches shown in Figure 6.

To see how the autoinhibition works with the used parameter values, the concentration field is given in Figure 7.



Figure 7: **Concentration field by autoinhibition;** Concentration field after 9000 MCS

Figure 7 shows the concentration field of the simulated model after 9,000 MCS. At the dark places, the concentration is the most high and becomes less how lighter the figure. At the arrows, the concentration is lighter than in the middle, so at these places is the concentration less and also the inhibition. So at the arrows there can be branching.

The compactness measured at 9,000 MCS is $C = 0.503 \pm 0.0179$. At 0 MCS the compactness is $C = 0.925 \pm 0.0062$. The decreasing of the compactness means that the relation between the area of the cluster and the area of the convex hull around the cluster is decreased, thus the cluster is no longer nearly convex, but contains branches.

The parameter values used in the results above are chosen to let branches occur. It is necessary to look at the different parameters one by one to study the sensitivity of the model to these parameters. This can be done by measuring the compactness and computing the morphospaces of parameters at different values.

3.1.1 Diffusion length

Study of the diffusion length shows that there is an optimal value of the diffusion length whereas the compactness is at its minimum. After 10 simulations the mean minimal compactness is $C = 0.370 \pm 0.0543$ at a diffusion length of $L = 2.236$. So at $L = 2.236$ the difference between the area of the cluster and its convex hull is at its maximum. Thus most of the branches occur in the cluster of cells, when $L = 2.236$.

To see if the diffusion length has any effect on the model it is necessary to compute the model with the constant at extreme values. Next a parameter sweep can be made to see what the sensitivity of the model according

to the diffusion length is. All figures in these section are made with the parameters values found in Table 1 on page 13, only the values of the diffusion length are given.

Let $L = 0$, then $L = \frac{D}{\varepsilon} = 0$, so $D = 0$ and thereby $D\nabla^2 c = 0$ in the partial differential equation of the concentration inhibitor. So this equation will be

$$\frac{\partial c}{\partial t} = \alpha(1 - \delta(\sigma(\vec{x}), 0)) - \varepsilon\delta(\sigma(\vec{x}), 0)c$$

where $\frac{\partial c}{\partial t}$ is only depending on the decay constant ε and the secretion constant α . That means that the concentration will rise during time, but the inhibitor will always stay in the pixels belonging to the epithelial cells. The concentration of inhibitor in pixels with cell type medium is zero, because there is no diffusion and secretion in medium, thus the effective energy component $\Delta H_{\text{inhibition}} = \mu c(\vec{x}') = 0$. This means that the pixels of cells are only inhibited to move in pixels of other cells, causing the cells to spread out to obtain the most favorable energy state.

Figure 8 shows the results when the diffusion length $L = 0$ and thus the diffusion constant $D = 0$.

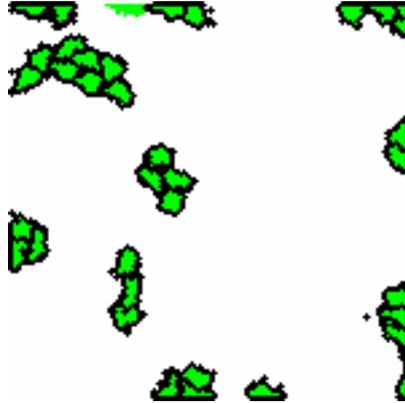


Figure 8: $L = 0$; Simulation state after 9000 MCS

The cluster from the beginning of the simulation is spread along the medium in a couple of clusters. The adhesion energy is strong enough to hold some cells together, but the initial cluster has fallen apart by the inhibition, just as expected.

On the other side it is interesting to see how the cells will move when the diffusion length is set to $L = \infty$. Then the diffusion constant is infinite, causing a high flow of inhibitor into the medium. Also the decay constant can be $\varepsilon = 0$, in such a way that the inhibitor will not be degraded and dif-

fuses into the medium. The concentration inhibitor in pixels of type $\tau = 0$, neighboring the epithelial cells, is now very high by the diffusion causing a positive change in the effective energy by copying a cell pixel into a medium pixel. The probability $e^{\frac{-\Delta H}{T}}$ will be very little because of the high ΔH . So no pixel of cell type EC will copy itself into medium pixels. That means that no cell will move outside the cluster into the medium. The initial shape will remain. Because the model can not work with infinity the constant will be set on $D = 1,000,000$. Figure 9 represents the results as L moves to infinity:

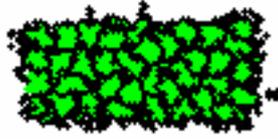


Figure 9: $L = \infty$; Simulation state after 9,000 MCS with $D = 1,000,000$

Figure 9 shows that the boundaries of the cells are not smooth anymore. The forward Euler method used for the differential equation has become numerically unstable for these high values of D . Another way to compute $L \rightarrow \infty$ is to set $\varepsilon = 1 \cdot 10^{-10}$. Figure 10 shows the results for $\varepsilon = 1 \cdot 10^{-10}$.



Figure 10: $L = \infty$; Simulation state after 9,000 MCS with $\varepsilon = 1 \cdot 10^{-10}$

Figure 10 shows the same results as with $D = 1,000,000$ but the boundaries are smoother. So forward Euler is less unstable here. The cluster has barely moved because of the high amount of inhibitor inside the medium.

The results of the simulations with the diffusion length at $L = 0$ and $L = \infty$ contain no branches, see Figure 8, Figure 9 and Figure 10. So the model is simulated 10 times around the value that give branches and the compactness is measured. The plot in Figure 11 shows the diffusion length against the compactness.

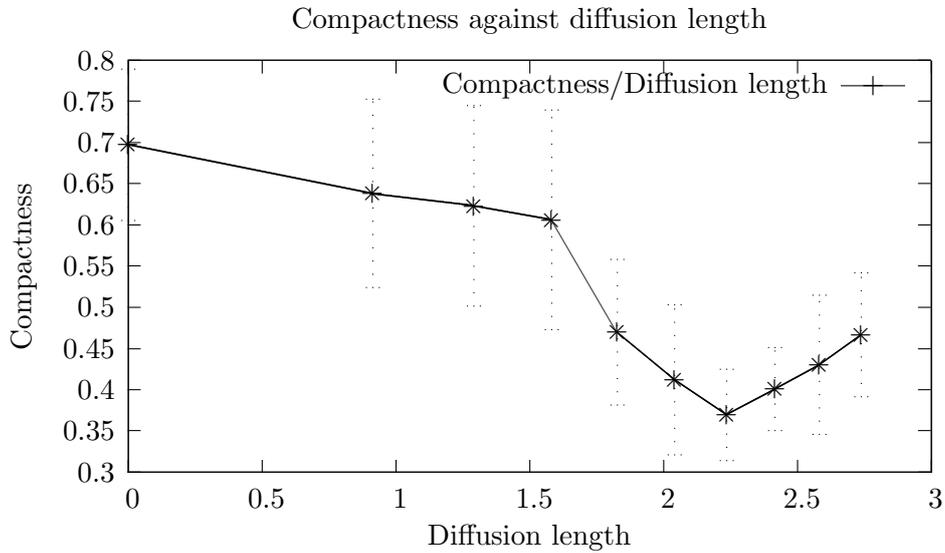


Figure 11: **Diffusion length against compactness;** Mean and standard deviations from 10 simulations at each value

The plot shows that when the diffusion length rises the compactness decreases. The concentration of c_1 will come further into the medium where it inhibits the branching of the epithelial cells. At $0 < L < 1.581$, there is a very low level of c_1 in the medium, so the cells can go everywhere, because there is no inhibition. The compactness is then measured over the largest cluster, but the initial cluster has fallen apart. When the diffusion length moves from $L = 1.581$ to $L = 2.236$, the concentration inhibitor inside the medium increases, inhibiting cells to move random and stay more and more together. Now the epithelial cells form branches, because at some spaces there is fewer concentration inhibitor. So the compactness decreases to a minimum at $C = 0.370$ for $L = 2.236$, but rises again from $C = 0.370$ to $C = 0.467$ for $L = 2.236$ to $L = 2.739$, where the diffusion length is long

enough to inhibit the epithelial cells to form any branches.

Besides the compactness, a morphospace also shows at which values of the diffusion length branches occur. Figure 12 shows the morphospace of the parameter sweep with the diffusion constant. The corresponding diffusion length can be found by using $L = \sqrt{\frac{D}{\varepsilon}}$.

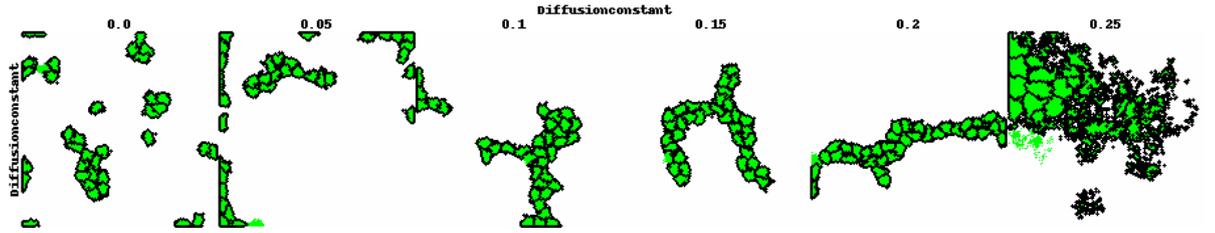


Figure 12: **Morphospace with different diffusion constants**; Cell field after 10,000 MCS

In the morphospace the same behavior as in the graph of the compactness is found. At low values of the diffusion length, from $L = 0$ to $L = 1.291$ (that is $D = 0.05$), the cells are spread through the medium and as the value rises, the cells form more and more a cluster with branches. The higher diffusion lengths, caused by higher diffusion constant values from $D = 0.25$, cause instability of the forward Euler method used for the differential equation. In figure 5, this instability is shown at the figure $D = 0.25$.

3.1.2 Temperature

Another interesting parameter is the temperature T . This is not the real temperature, but a non-physical temperature describing the cell motility, in that way that a higher temperature stands for a stronger cell motility. The movements of epithelial cells is driven by extension of the pseudopodia of a cell. This mode of motility is similar to the crawling movement of a single cell. On the lattice at the CPM, one pixel copy itself into another with a probability $e^{-\frac{\Delta H}{T}}$ if $\Delta H > 0$. Changes in the temperature thus effect the copy probability with positive ΔH , with higher temperatures causing higher probabilities because $\frac{-\Delta H}{T}$ moves to zero when T moves to infinity, so $e^{-\frac{\Delta H}{T}}$ moves to 1.

Study shows that the temperature effects the rate of movement of the cells. When $T = 0$ the cells will not move at all, but branches occur at $T \geq 50$ until $T = 200$.

As with the diffusion length, we start by studying the effect of the tem-

perature on the model, by computing extreme values, namely $T = 0$ and $T \rightarrow \infty$. After that a parameter sweep can be done to study the sensitivity of the model due to the temperature. All figures in these section are made with the parameters values found in Table 1 on page 13, only the values of the temperature are given.

Set $T = 0$, then $\frac{-\Delta H}{T} = -\infty$ such that $e^{\frac{-\Delta H}{T}} = 0$. Thus when the temperature is zero the probability that a pixel will copy itself into another, causing a positive energy change, is zero. These copies will not exist. The copies of cell pixels into medium pixels are accompanied with positive energy changes. So these copies are not present in the model with $T = 0$. Copies into a direction with negative energy change can occur, but only at pixels of cells. That means that all cells will stay where they began, because to move into a spheroid, the most favorable energy state, pixel with $\tau = 1$, cell type epithelial, have to copy into pixels with $\tau = 0$, cell type medium, which is not possible at $T = 0$. Thus the cell motility is zero. Figure 13 will show the results with $T = 0$.

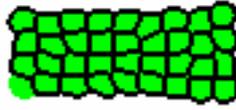


Figure 13: $T = 0$; Simulation state after 9000 MCS

The cluster in Figure 13 is after 9,000 MCS still in the same position as it was at the beginning, meaning that the cells did not move at all. That was precisely what has been expected.

Now set the temperature $T = \infty$. Then $\frac{-\Delta H}{T} = 0$, so $e^{\frac{-\Delta H}{T}} = 1$. Thus

$$P(\Delta H) = \begin{cases} 1 & \text{if } \Delta H \geq 0 \\ 1 & \text{if } \Delta H < 0 \end{cases}$$

That means that it does not matter whether a pixel tries to copy itself with a positive energy change or a negative energy change, in both cases the copy will always succeed. The cells will spread among the medium. Figure 14

shows the results with $T = 1,000,000$, because the model can not work with infinity.



Figure 14: $T = \infty$; Simulation states after (A) 1,000 MCS and (B) 9,000 MCS and $T = 1,000,000$

The pixels with cell type medium will copy themselves in other pixels with probability 1. At this way a complete cell can disappear because medium pixels copied themselves into the cell pixels. And once a cell is lost, it cannot come back again (see also Voss-Böhme (2012)). Because the high amount of medium around the initial cluster of cells, the cells are almost disappear at 1,000 MCS and totally disappeared at 9,000 MCS, see Figure 14.

At $T = 0$ there is no movement and at $T = \infty$ the cell motility is so high that all the cells are replaced by medium. Therefore a parameter sweep is done around the value $T = 150$ in the example in Figure 6 on page 14 and the compactness is measured and plotted against the temperature values in figure 15.

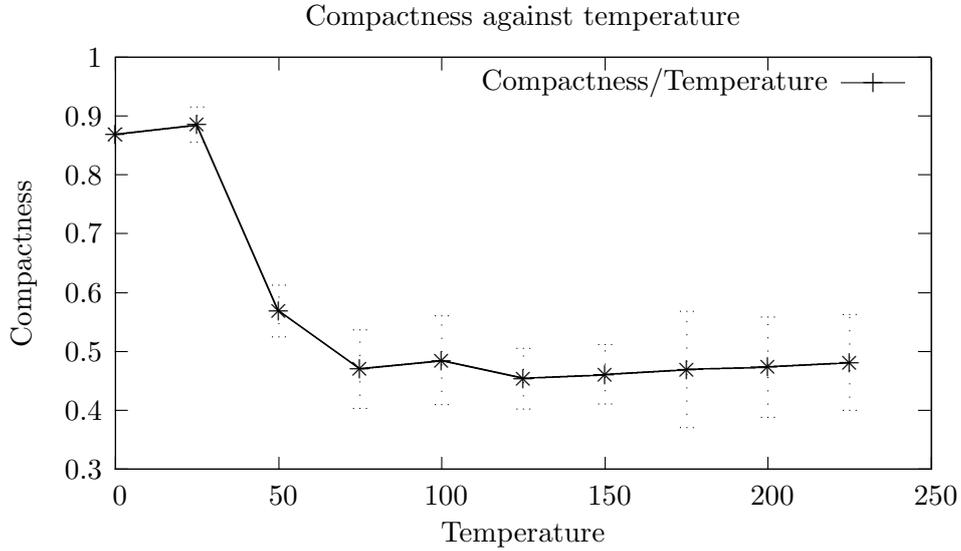


Figure 15: **Temperature against compactness**; Mean and standard deviations from 10 simulations at each value

The plot in Figure 15 shows different values of the temperature against the compactness. A high drop occurs between temperatures $T = 25$ and $T = 75$. The meaning of this drop can be found in the morphospace in Figure 16.

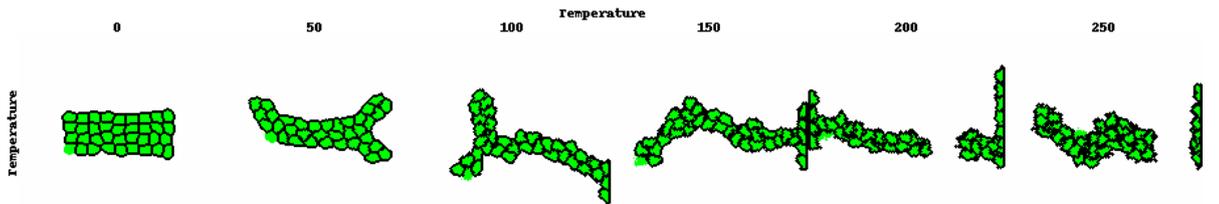


Figure 16: **Morphospace with different temperatures**; Cell field after 10,000 MCS

The epithelial cells are in initial state at temperature $T = 0$. This because the cells can only move into directions with negative energy change when $T = 0$, but the movements into these directions are only into the cluster, never outwards. There is no movement outwards because such a movement causes more adhesion energy and surface and therefore always a positive energy change, in which direction a cell can not move. So there will be no movement at all.

At $T = 50$ the probability $e^{-\frac{\Delta H}{T}}$ is high enough to cause copies into directions with positive energy change. Thus a cell can move outwards the cluster and branches can occur by inhibiting neighboring cells to move outwards, causing the compactness to drop. At $T \rightarrow \infty$ the probability of movements at positive energy changes will be almost 1, so that the copy probability in both positive and negative directions of the energy will be the same and so the cells will move randomly. Movement not longer depends on inhibition.

3.1.3 Inhibition constant

The last parameters that will be discussed is the inhibition constant μ . Study of the inhibition constant shows that this constant effects the ability to form branches. When the inhibition constant $\mu = 0$, the initial cluster will form a spheroid, whereas $\mu = \infty$ the initial cluster is almost the same after 10,000 MCS. Figure 19 shows that $\mu = 600$ is the mean extreme value that gives a minimum compactness of $C = 0.260 \pm 0.0245$. At $\mu = 600$ the most branches occur.

Again we study the effect of the inhibition constant on the model, by first taking extreme values, namely $\mu = 0$ and $\mu = \infty$. After that a parameter sweep can be done to study the sensitivity of the model according to the inhibition constant. All figures in these section are made with the parameters values found in table 1 on page 13, only the values of the inhibition constant are given.

Set $\mu = 0$. Then $\Delta H_{inhibition} = \mu c(\vec{x}') = 0$ for every \vec{x} in the lattice. So the effective energy only change depending of the adhesion energies and surfaces of the cells. The model has become the standard CPM. The CPM will always go to the most favorable energy state: the spheroid. Here are the least boundaries and the smallest surfaces, that gives the lowest result of the Hamiltonian, because then the adhesion energy and the volumeconstraint of the Hamiltonian are that low, that further decreasing of the constraints causes a positive ΔH . Figure 17 shows the results with $\mu = 0$.



Figure 17: $\mu = 0$; Simulation state after 9000 MCS

The cluster starts to form a spheroid in which its most favorable energy state is reached. After 9000 MCS the model is not yet in its minimal energy state, so the cluster is not yet a spheroid.

Set $\mu = \infty$, then the inhibition component of the Hamiltonian, ΔH , will be $\Delta H = \mu c(\vec{x}') = \infty$. So the effective energy change is infinite everywhere on the lattice. An infinite energy change gives the probability of a copy into a direction with positive ΔH to be $\lim_{T \rightarrow \infty} e^{-\frac{\Delta H}{T}} = 0$. In every direction is the $\Delta H = \infty$, so no copy will succeed. The initial cluster will remain the same. Figure 18 shows the results when $\mu = \infty$.



Figure 18: $\mu = \infty$; Simulation state after 9000 MCS with $\mu = 1,000,000$

Now the inhibition is very strong and so it inhibits the cells to move. The initial cluster will stay, and because the model cannot work with $\mu = \infty$, the probability for a cell to move is not zero. So the cells move very close

around their initial position.

To find a proper value of the inhibition constant where branches can occur, a parameter sweep is done and the compactness is measured, see Figure 19.

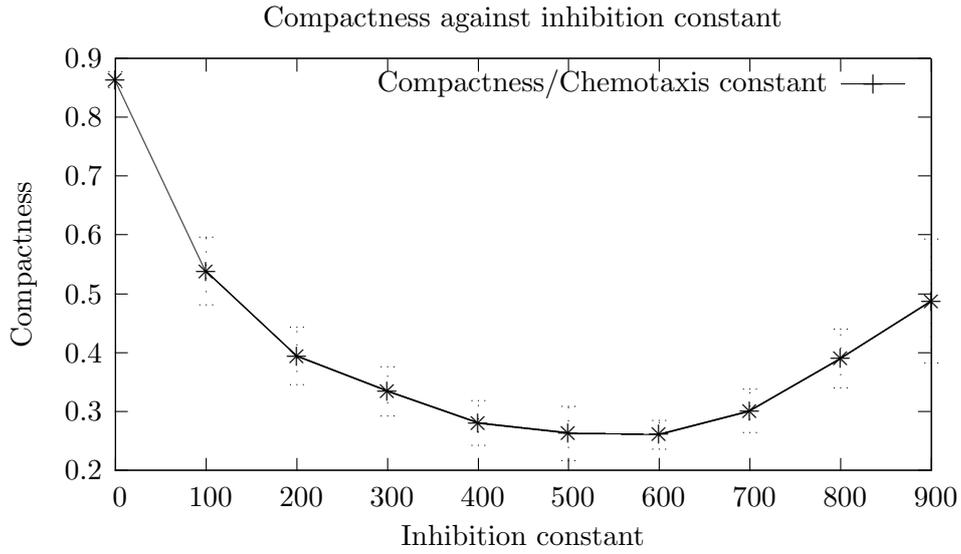


Figure 19: **Inhibition constant against compactness;** Mean and standard deviations from 10 simulations at each value

The graph for the inhibition constant against the compactness shows that there exists an optimum value of $\mu = 600$ where the compactness is at its minimum, namely at $C = 0.260$. So at $\mu = 600$ the most branches should appear. To check this value a morphospace is made to see if the cells stay together or if they scatter. Figure 20 shows this morphospace.

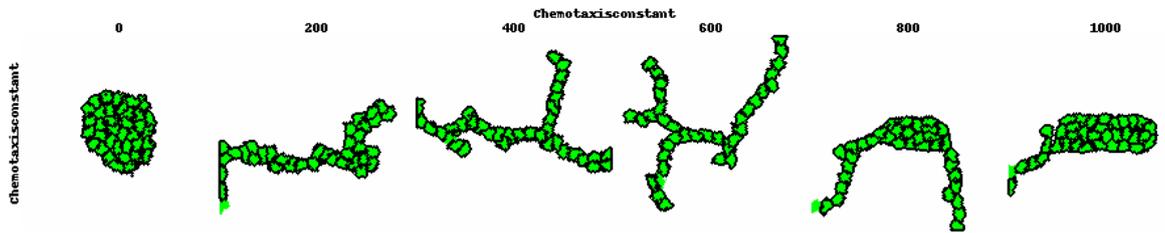


Figure 20: **Morphospace with different inhibition constants, from 0 to 1000; Cell field after 10,000 MCS**

The first drop in the plot shown in figure 19 can be explained by the fact that when the inhibition constant is zero, there is no inhibition and so the cells move to the lowest energy state, a spheroid, as reflected by the compactness close to $C = 1$. For values of μ closer to $\mu = 100$, the inhibition component of the Hamiltonian will be positive, so the ΔH can become positive, causing a probability between 0 and 1 that copies of pixels with a positive ΔH will succeed. The epithelial cells will move into directions with the lowest positive energy change, because these directions have the highest probability. The cells that move outwards the cluster inhibits the neighbor cells, so branches occur.

There is an optimal value of $\mu = 600$. When the constant is higher than $\mu = 600$, the ΔH will become too high and the probability of a copy will become very low. A copy will almost only occur when the energy change of a movement is negative. But this movement is very rare, because of the inhibitor around the cells. That explains the rise of the compactness in the plot (Figure 19) and the near absence of branches in the morphology when the inhibition constant has value 1000.

The plot shown in figure 19 shows a steep drop of the compactness between $\mu = 0$ and $\mu = 100$. To see if there is a particular value of μ where the compactness starts to drop the compactness of the clusters with the inhibition constant between $\mu = 0$ and $\mu = 100$ is measured. Figure 21 shows the graph of the compactness against the inhibition constant, zoomed in on $\mu = 0$ till $\mu = 100$.

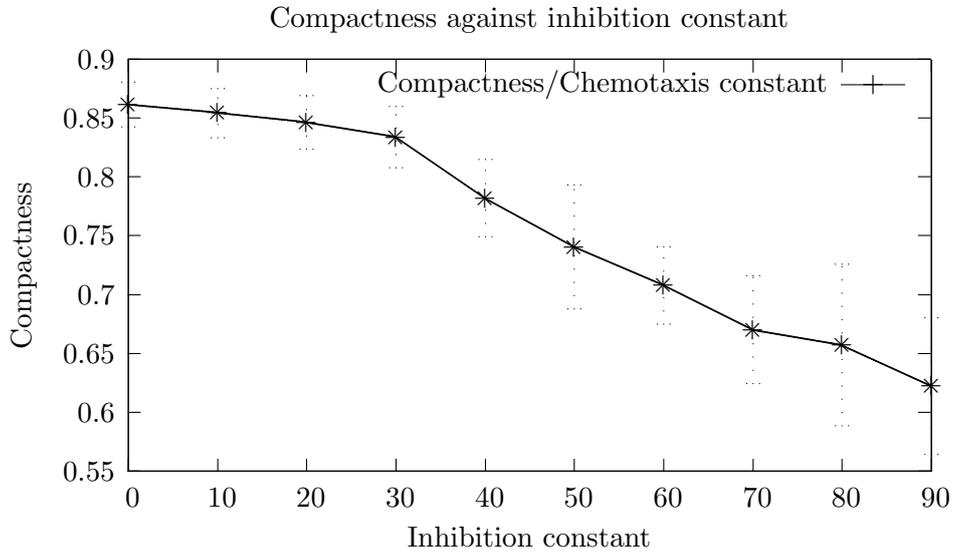


Figure 21: **Inhibition constant against compactness**; $\mu = 0$ till $\mu = 100$. Mean and standard deviations from 10 simulations at each value

Zoomed in on the first 100 steps of the inhibition constant, the graph in Figure 21 shows a relatively smooth decrease of the compactness. So there is not a specific value of the inhibition constant that causes a steep decrease of the compactness. Its decrease is stronger between $\mu = 0$ and $\mu = 100$ than between $\mu = 100$ and $\mu = 600$, but smooth.

A morphospace is also made to see what happens between $\mu = 0$ and $\mu = 100$. This morphospace is found in Figure 22.

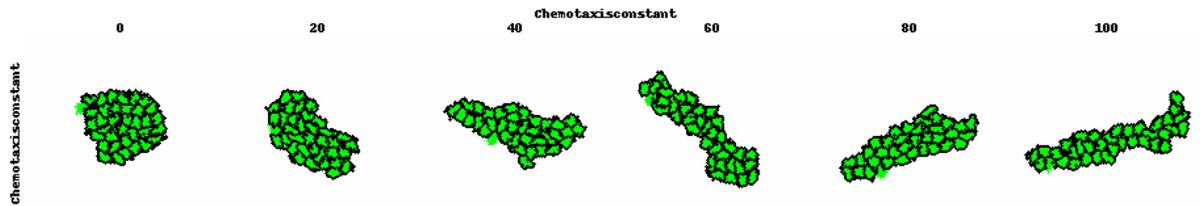


Figure 22: **Morphospace with different inhibition constants, from 0 to 100**; Cell field after 10,000 MCS

The morphospace also shows no big changes between the morphologies. The initial cluster of cells become longer and flatter when the inhibition constant moves to $\mu = 100$, but in a smooth way and not at a specific value

of μ .

3.1.4 Conclusion

The inhibition model shows that branches occur when the parameters of the model are set as in table 1. We studied the dependence of the branching mechanism on the diffusion length, the temperature and the inhibition constant. The diffusion length has an optimal value at $L = 2.236$ for which the compactness is at its minimum $C = 0.370$ and the most branches occur. The temperature does not have an optimum, the compactness makes a steep drop at $T = 50$ but remains the same at temperatures higher than $T = 50$. At $T = 200$ the cluster of cells falls apart so the compactness can stay the same, but the branches disappear. The temperature, thus the cell motility, effects the adhesion of the cells. At $T < 50$ the cells stay to close together to form any branches and at $T > 200$ the cell motility is to high to remain the initial cluster of cells. The inhibition constant has again an optimum for which the compactness is minimal, namely $C = 0.260$. This optimum is at $\mu = 600$, the value where the most branches occur.

3.2 EC-Mesenchym model

The experiments and computations done by T. Hirashima showed that branches occur when the sources of growth factor are set on fixed point and the epithelial tissue is elastic.

To make a more realistic model, the Epithelial-Mesenchymal Model is made to simulate the appearance of branches when the epithelial cells form a autocrine loop with the mesenchymal tissue around the cells. In this loop, the epithelial cells produce a substance c_1 . This substance activates the mesenchymal cells to produce more of a substance c_2 to which the epithelial cells chemotact. Besides that the elasticity of the epithelial tissue is gone.

In this section, the results of the second model, the epithelial cells inside mesenchymal tissue will be given. First an example will be given to see how the model works and if branches occur. The parameter values for this example will be given in that way that branches occur. To study the sensitivity of the model according to the parameters different parameter sweeps will be done for the diffusion length, the temperature and the chemotaxis constant. Each of these parameters will be discussed to see what the behavior is on the extreme values of zero and infinity, because negative values will not be found in nature.

To begin the EC-Mesenchym model is computed with given values of the parameters to get an example with branches. The values of the parameters are given in table 2 An example of this model is given by the following

parameters:

Table 2: Parameter values in computation of EC-Mesenchym Model

Parameter	Cell type	Value
Temperature		20
Adhesion energy	Medium - EC	0
	Medium - Mesen	0
	EC - Mesen	8
	EC - EC	1
	Mesen - Mesen	10
Chemotaxis constant	c_2	150
Secretion constant	c_1	0.13
	c_2	0.0001
Diffusion constant	c_1	0.24
	c_2	0.24
Decay constant	c_1	0.003
	c_2	0.02

The adhesion energy between medium and the epithelial cells and between the medium and the mesenchymal cells is set to zero for the same reason as in the Inhibition Model.

Figure 23 shows the initial cluster of epithelial cells inside the mesenchymal tissue.

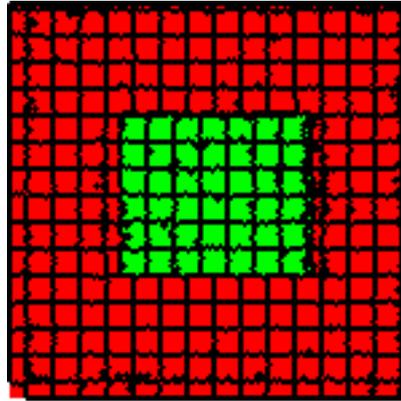


Figure 23: **Autocrine loop between epithelial and mesenchymal cells**; Simulation state after 0 MCS

Figure 23 contains both red and green cells. The red ones are the mesenchymal tissue, that surrounds the green epithelial cells, such that it is the same as in the beginning of branched organs.

Now the model is computed with the given parameters values and is given in Figure 24.

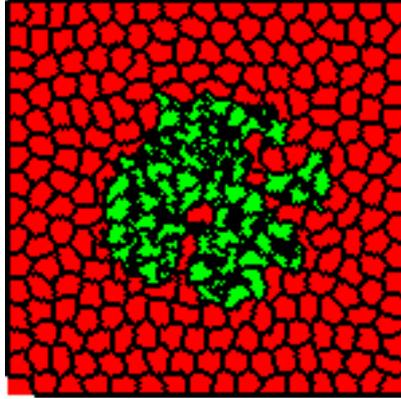


Figure 24: **Autocrine loop between epithelial and mesenchymal cells**; Simulation state after 1250 MCS

Figure 24 shows that the epithelial cells are moving inside the mesenchymal tissue. The epithelial cells produce the substance c_1 , that diffuses into the mesenchymal tissue. The tissue reacts by producing the substance c_2 . This c_2 reaches the epithelial cells and causing a chemotaxis towards the c_2 . In that way, the epithelial cells start moving inside the tissue. At some points even branches occur, because some epithelial cells are stronger attracted to the c_2 than their neighbors. But on other points on the lattice the attraction is stronger than the adhesion energy and the cells are moving away from the cluster and don't even have contact anymore.

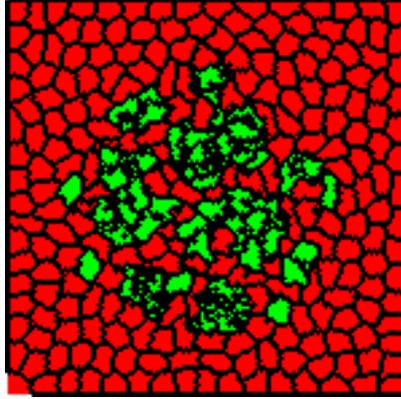


Figure 25: **Autocrine loop between epithelial and mesenchymal cells**; Simulation state after 2750 MCS

When the model runs longer to 2750 MCS, all the cells spread through the mesenchymal tissue (see Figure 25).

The compactness measured at 1250 MCS is $C = 0.803 \pm 0.0201$ after ten simulations against at compactness of $C = 0.947 \pm 0.0043$ at 0 MCS. So the convex hull around the surface of the epithelial cluster is bigger at 1250 MCS, and at 1250 MCS, the initial cluster is still complete, so there are some branches. At 2750 MCS is the compactness $C = 0.683 \pm 0.0606$, a little bit lower than at 1250 MCS. Looking at Figure 25, the initial cluster has fallen apart in some sub-clusters. The compactness of the biggest sub-cluster is then measured, what can cause a low compactness if the cluster contain some branches, but there are no branches in the initial cluster.

The parameter values used in the results above are chosen to let branches occur. It is necessary to look at the different parameters one by one to study the sensitivity of the model according to these parameters, in this case the diffusion length, the temperature and the chemotaxis constant. This can be done by measuring the compactness and computing the morphospaces of the parameters at different values.

3.2.1 Diffusion length

In the Epithelial-Mesenchymal Model both the substances c_1 and c_2 have diffusion, so also both the substances have a diffusion length. These lengths will be discussed separately here because they both have an other effect on the model.

Study of the diffusion length of c_1 shows that at $L = 0$ the model is the

same as the CPM and so the epithelial cluster will form a square inside the mesenchymal tissue. For $0 < L < 2.887$ the compactness rises from $C = 0.915 \pm 0.0117$ to $C = 0.932 \pm 0.573$. For $2.887 < L < 8.660$ the compactness decreases to $C = 0.795$ and branches are found. At $L = \infty$ the epithelial cluster will fall apart due to chemotaxis.

Study of the diffusion length of c_2 shows that at $L = 0$ the model again works as the CPM and so a epithelial square will be formed. For $0 < L < 1.118$ the compactness decreases from $C = 0.876$ to $C = 0.524$. For $1.118 < L < 3.354$ the compactness rises again to $C = 0.747$ and branches are found. At $L = \infty$ the epithelial cells will form a spheroid.

We will first study the effect of the diffusion length on the model at the extreme values $L = 0$ and $L = \infty$. After that a parameter sweep can be done to study the sensitivity of the model for the diffusion length. All figures in these section are made with the parameter values found in Table 2 on page 29. The values of the diffusion length differs through this section and are given at the figures.

Set the diffusion length of c_1 to $L = 0$. According to section 3.1.1 the diffusion constant $D = 0$ when $L = 0$, so there is no diffusion of c_1 into the mesenchymal tissue. So there is no activation of the mesenchymal tissue to produce c_2 . Thus there is a very little amount of c_2 present, but this is too low to let the epithelial cells chemotact. Therefore the model will go to form a square of epithelial cells inside the mesenchymal tissue, its most favorable energy state because then the sum of boundary energies and surface energies is at its lowest point. Figure 26 shows the results when $L = 0$ for substance c_1 .

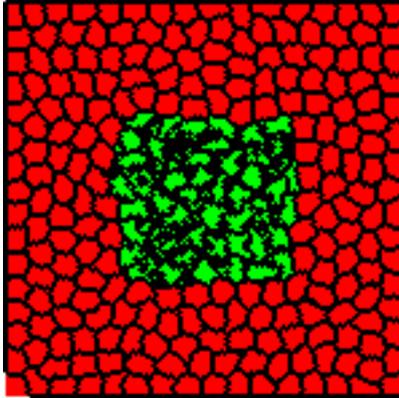


Figure 26: $L = 0$; Simulation state after 1250 MCS and L of substance c_1

The initial cluster of epithelial cells moved into a square inside the mesenchymal tissue in Figure 26. This is its most favorable energy state, just as expected.

Set $L = \infty$ of substance c_1 , then two different situations can exist. The first one is that the diffusion constant of c_1 is $D = \infty$, causing a uniform concentration of c_1 on the lattice, because $\frac{\partial c_1}{\partial t}$ is the sum of all the production rates in the cells. Then all mesenchymal cells will produce c_2 at the same rate and thus the chemotaxis is no longer dependent on the local geometry. So the cells will spread over the lattice. On the other side, the decay constant $\varepsilon = 0$ when $L = \infty$. Then the concentration c_1 will also become uniform, causing a spread of the epithelial cells. Figure 27 shows the results when $L = \infty$.

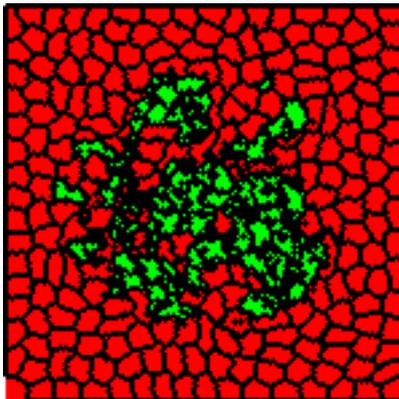


Figure 27: $L = \infty$; Simulation state after 1250 MCS and $\varepsilon = 1 \cdot 10^{-10}$

Figure 27 shows that the epithelial cells are spread through the mesenchymal tissue. However the concentration c_1 is not uniform, but there are still some little gradients. It will take some time before the gradients settle down. Only when $L = \infty$ the production rate of c_2 is uniform, but this can not be tested by simulations.

After studying the extreme values of the diffusion length of c_1 , a parameter sweep is done to find proper values where branches occur. The compactness is also measured and given in Figure 28.

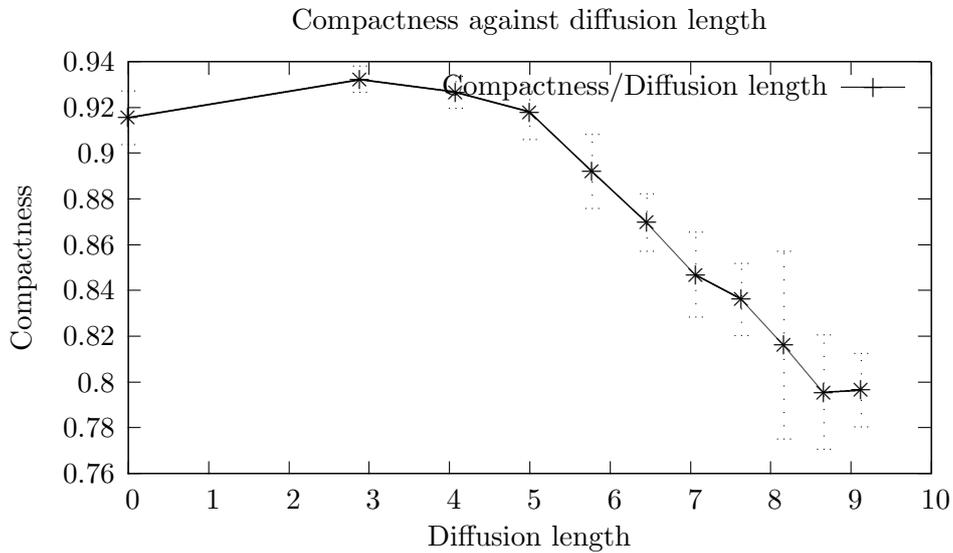


Figure 28: **Compactness against diffusion length;** Mean and standard deviations from 10 simulations at each value

Figure 28 shows that the compactness rises from $C = 0.915 \pm 0.0117$ to $C = 0.932 \pm 0.0117$ when the diffusion length rises from $L = 0$ to $L = 2.887$. The substance c_1 diffuse further into the mesenchymal tissue, thereby activating more mesenchymal cells to produce more c_2 . Thus for $L > 0$ there is chemotaxis. For $0 < L < 2.887$ the diffusion of c_1 activates only the neighboring mesenchymal cells to produce more c_2 . So the epithelial cluster will become a spheroid because every cell chemotact to the neighboring mesenchymal cells. Therefore the compactness rises. For $L > 2.887$ the c_1 goes far enough into the mesenchymal tissue to activate those cells for producing more c_2 . So at some points the chemotaxis is stronger then on others, and thus branches can occur and the compactness decreases. At

$L = 9.129$ the value of the diffusion constant is that high that instability occur in Euler Forward. For $L \rightarrow \infty$ the gradient of the concentration will become flat, because of the open boundary condition. Then the chemotaxis does not longer react on local gradients and thus the cells spread out. Then the compactness will be high because it will be measured over the biggest sub-cluster of cells.

A morphospace for the values of the diffusion constant is made in order to see how the diffusion length affects the cellfield (see Figure 29).

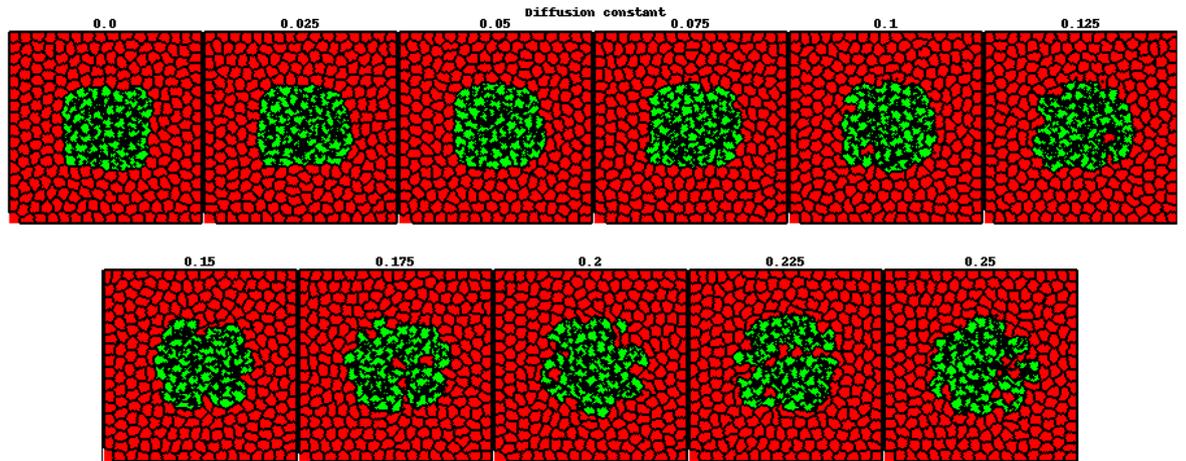


Figure 29: **Morphospace with different diffusion constant;** Cell field after 1250 MCS

In the morphospace in Figure 29 the same behavior can be found. For $0 < D < 0.025$, that is $0 < L < 3.162$, the epithelial tissue becomes a spheroid and for $D > 0.025$ the epithelial cells performs stronger chemotaxis and branches occur, so the compactness decreases, until $D = 0.25$ where instability occurs in Euler Forward.

Secondly, set the diffusion length of c_2 at $L = 0$. That means that $D = 0$ of c_2 , so there is no diffusion of c_2 into the epithelial cells. Thus there is no chemotaxis of the epithelial cells and the model will form a square of epithelial cells within the mesenchymal tissue. Figure 30 will give the result of $L = 0$.

The same thing happens in Figure 30 as when the diffusionconstant of c_1 was set at $L = 0$ (see Figure 26). There is no chemotaxis and the model brings itself in the energetic most favorable state.

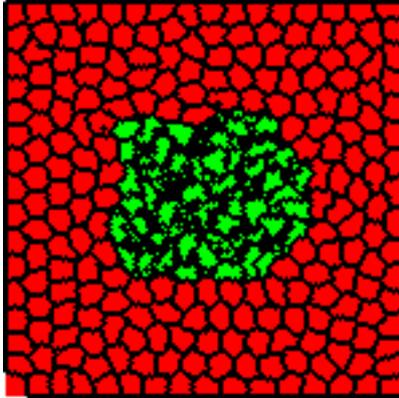


Figure 30: $L = 0$; Simulation state after 1250 MCS

Set $L = \infty$, then again there are two situations. Either $D = \infty$, so c_2 is diffused very strong into the epithelial cells causing a strong chemotaxis of the epithelial cells and therefore a strong spread of these cells. Or $\varepsilon = 0$, so there is no decay of c_2 what causes also a strong chemotaxis and therefore a spread of the epithelial cells. Figure 31 shows the results when $L = \infty$.

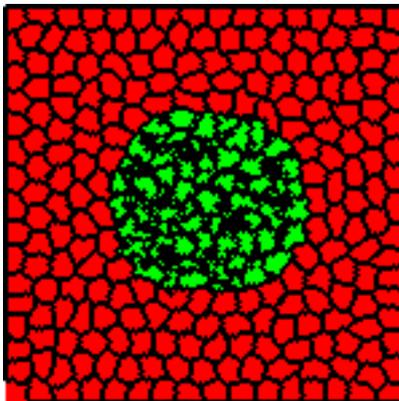


Figure 31: $L = \infty$; Simulation state after 1250 MCS and $\varepsilon = 1 \cdot 10^{-10}$

The expectation was to find that the epithelial cells were all spread through the mesenchymal tissue in Figure 31 due to the chemotaxis. Now $L = \infty$ so the gradients of the concentration c_2 will settle down when the time goes to infinity. Thus the epithelial cells will all chemotact to its neighboring mesenchymal cells. So the cluster will become a spheroid. After studying the extreme values of the diffusion length of c_2 , a parameter

sweep is done to find proper values where branches occur. The compactness is also measured and given in figure 32.

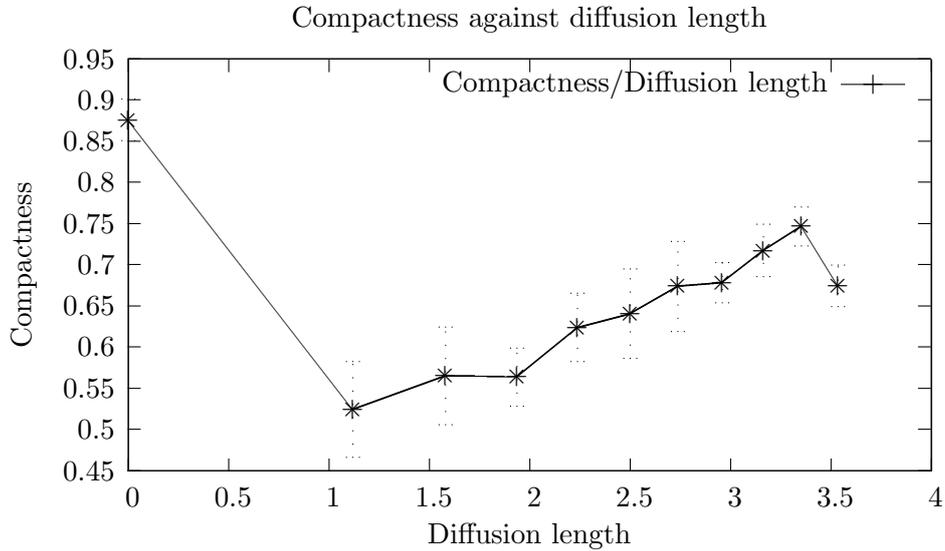


Figure 32: **Compactness against diffusion length;** Mean and standard deviations from 10 simulations at each value

Figure 32 shows when the diffusion length moves from $L = 0$ to $L = 1.118$ the compactness drops from $C = 0.876$ to $C = 0.524$. When the diffusion length is $0 < L < 1.118$ the substance c_2 will not diffuse far into the mesenchymal tissue. So there is a strong chemotaxis of the epithelial cells to c_2 . Individual cells can react stronger on c_2 than others, so the cluster will scatter. The compactness becomes this low because some sub-clusters are flattened, but the initial cluster is fallen apart. For $1.118 < L < 3.354$ the compactness rises from $C = 0.524$ to $C = 0.747$ because c_2 further into the mesenchymal tissue and so the chemotaxis is less strong. Therefore the initial cluster will not fall apart and even branches can occur. In the model the diffusion length is measured by changing the diffusion constant, so at $L = 3.536$ the diffusion constant is that high that the model gets negative concentrations at the Euler Forward method and so instability occur. The same behavior can be found in the morphospace in Figure 33.

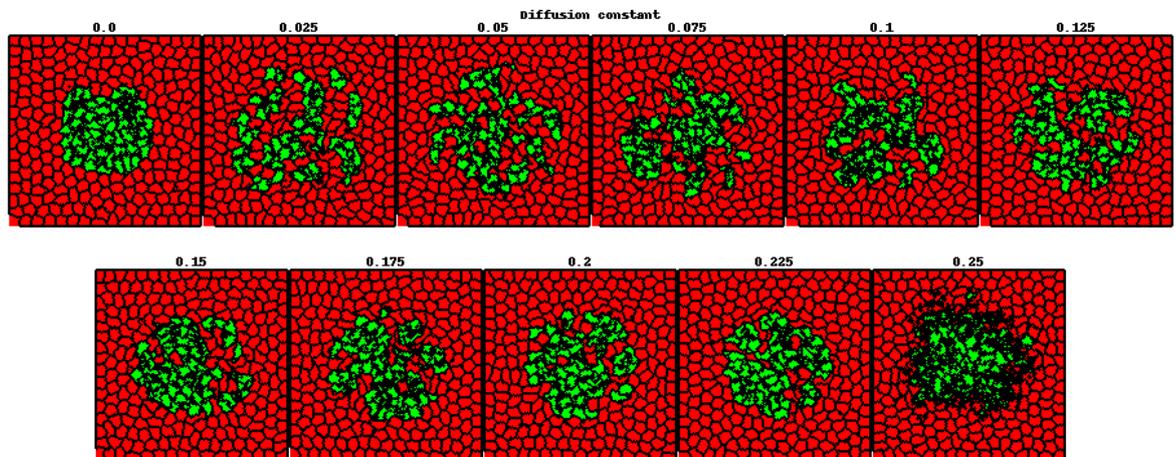


Figure 33: **Morphospace with different diffusion constant;** Cell field after 1250 MCS

The epithelial cluster in Figure 33 belonging to the diffusion constant value of $D = 0.025$ is scattered through the mesenchymal tissue due to the strong local chemotaxis. For $0.025 < D < 0.25$ less sub-clusters appear for higher D . At $D = 0.25$ the instability can be found.

3.2.2 Temperature

In the same way as in the Inhibition Model here the temperature is not a physical temperature but a 'cellular' temperature representing the cell motility. High cellular temperatures indicate high random cell motility.

Study of the temperature shows that rate of movement is effected by this parameter. For $T = 0$ the cells can only move when the energy lowers by their movements. For $0 < T < 20$ the compactness drops steeply but then remains the same for $20 < T < 180$. At $T = \infty$ cells will fall apart and spread over the medium.

We first study the effect of the temperature on the model at the extreme values $T = 0$ and $T = \infty$. After that a parameter sweep can be done to study the sensitivity of the model according to the temperature. All figures in these section are made with the parameter values found in Table 2 on page 29. The values of the temperature differs through this section and are given at the figures.

Set $T = 0$, then the probability of a copy into the direction with a positive ΔH is zero (see section 3.1.2), so these copies will not exist. The movement by chemotaxis is caused by a negative ΔH , because in the formula

$$\Delta H_{\text{chemotaxis}} = -\mu \left(\frac{c(\vec{x}')}{1 + sc(\vec{x}')} - \frac{c(\vec{x})}{1 + sc(\vec{x})} \right)$$

the energy change $\Delta H_{\text{chemotaxis}} < 0$ if \vec{x}' contains a higher concentration chemoattractant than \vec{x} , so there still will be chemotaxis. Besides chemotaxis the model also computes the state with the least energy H of the Hamiltonian. Figure 34 shows the result with $T = 0$.

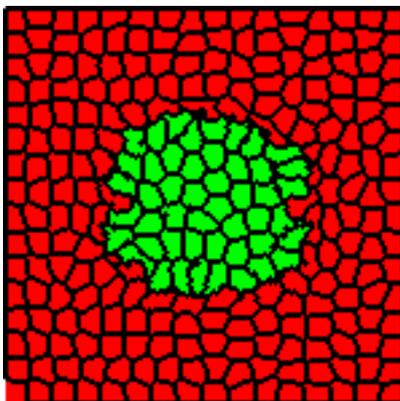


Figure 34: $T = 0$; Simulation state after 1250 MCS

Figure 34 shows that the epithelial cells are slightly moved in the mesenchymal tissue. The spheroid occurred by computing the most favorable energy state, but also some branches can be found in Figure 34, formed by the chemotaxis of the epithelial cells. The secretion and diffusion of the substances is still active, also the chemotaxis. With more steps, the cells are spread along the tissue and the initial cluster no longer exists.

Set now $T = \infty$, then the probabilities of the copies in direction of both positive and negative energy change are 1 (see section 3.1.2). A pixel then moves into every direction with the same probability and thus all the pixels will be spread over the lattice. In Figure 35 the results for $T = \infty$ are given.

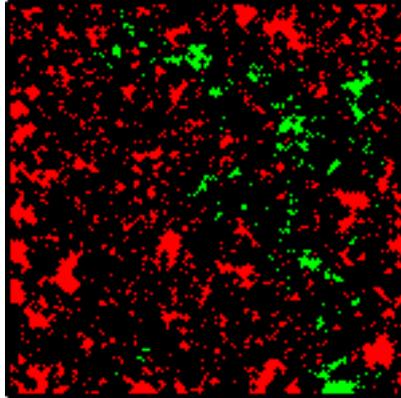


Figure 35: $T = \infty$; Simulation state after 1250 MCS with $T = 1,000,000$

Figure 35 shows a black plane with some red and green dots. The red dots are the mesenchymal cells and the green ones the epithelial cells. The black plane is made of boundaries between the different pixels of cells. The pixel of one cell moves to everywhere with the same probability, so pixels with the same cell type can be lying apart from each other. At that way one cell can be split into two or more cluster of pixels with the same cell type. Each of these clusters has a boundary (in black) causing the high amount of black in the picture. So at a temperature of $T = \infty$ the temperature is so high that even cells does not stay together anymore.

After studying the extreme values of the temperature, a parameter sweep is done to find proper values where branches occur. The compactness is also measured and given in Figure 36.

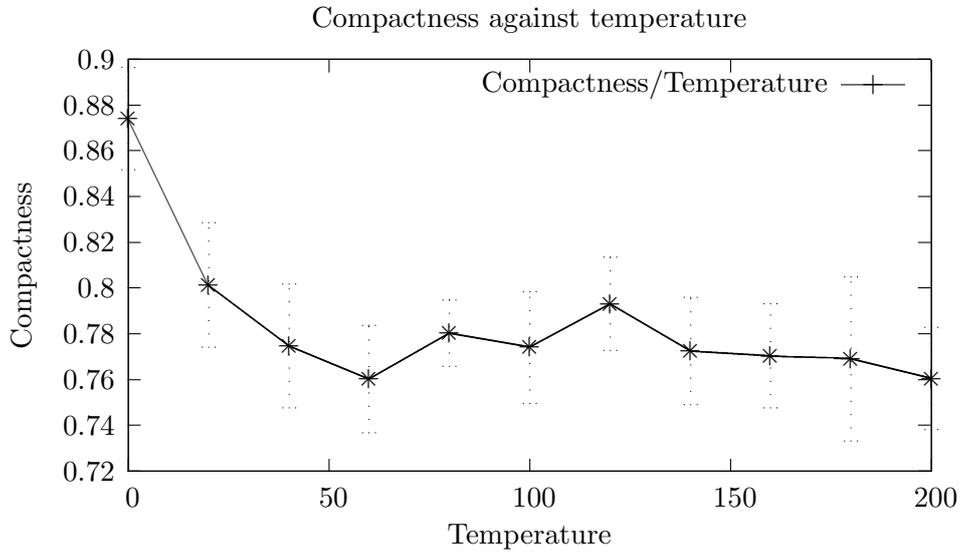


Figure 36: **Compactness against temperature;** Mean and standard deviations from 10 simulations at each value

The graph in Figure 36 shows a steep drop of $C = 0.881$ to $C = 0.776$ between $T = 0$ and $T = 20$. After $T = 20$ the compactness remains approximately the same, looking at the errorbars of the found compactness values. So the temperature is important only at low levels. After $T = 20$ the temperature does not effect the compactness anymore until $T = 180$. Branches occur from $T = 0$, because the initial cluster has a compactness of $C = 0.949 \pm 0.00731$ and so at $T = 0$ the compactness is decreased by 0.068. So to let any branches occur at higher speed it is necessary to set $T \geq 20$. The same behavior as in the graph can be found in the morphospace of the temperature. Figure 37 shows this morphospace.

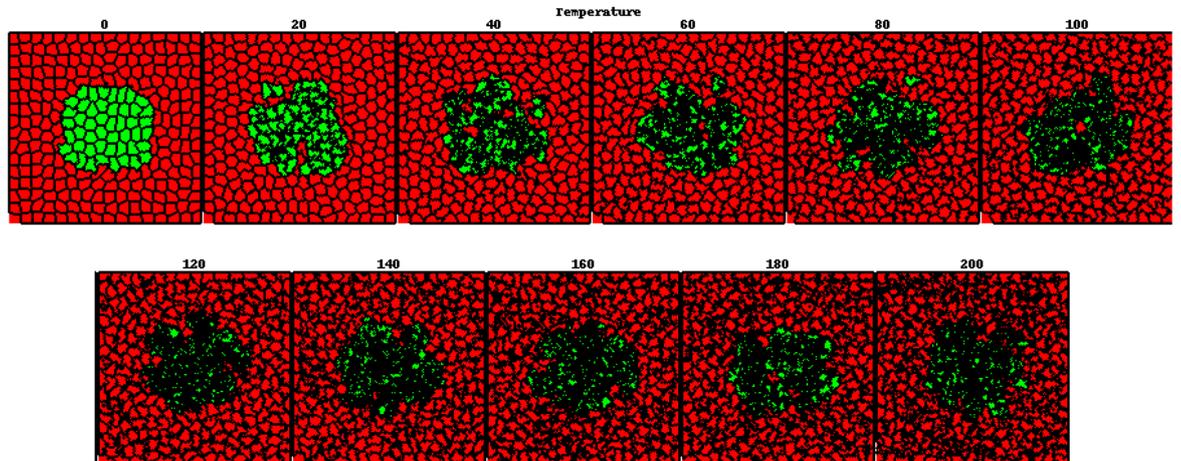


Figure 37: **Morphospace with different temperatures;** Cell field after 1250 MCS

The morphospace in Figure 37 shows that some branches occur for $T \geq 20$. At $T = 0$ the cluster gets a notch and the epithelial cells starts to form some branches, but this process is slower than for $T \geq 20$. At $T = 80$ the figure starts to be more and more black. So for $T > 80$ more boundaries are formed in the same way as discussed for $T = \infty$.

3.2.3 Chemotaxis constant

The last parameter to be discussed here is the chemotaxis constant. In the Epithelial-Mesenchym Model the substance c_1 does not have a chemotaxis constant, because there is no chemotaxis to c_1 . Substance c_1 only activates the mesenchymal cells to produce the substance c_2 to which the epithelial cells chemotact. Therefore in the following text is by chemotaxis constant the chemotaxis constant of substance c_2 meant.

Study of the chemotaxis constant of substance c_2 shows that at $\mu = 0$ there is no chemotaxis and the model finds its most favorable energy state. For $0 < \mu < 50$ this state becomes a spheroid of epithelial cells inside the mesenchymal tissue and for $\mu \geq 50$ the cluster begins to show branches and even falls apart at $\mu = 250$. For $\mu = \infty$ the chemotaxis is that strong that all the mesenchymal cells will disappear.

It is necessary to study the effect of the chemotaxis constant on the model at the extreme values $\mu = 0$ and $\mu = \infty$. After that a parameter sweep can be done to study the sensitivity of the model according to the chemotaxis constant. All figures in these section are made with the parameter values

found in Table 2 on page 29. The values of the chemotaxis constant differs through this section and are given at the figures.

Set $\mu = 0$, then $\Delta H_{\text{chemotaxis}} = 0$. That means energy change over the lattice of the CPM only depends on the Hamiltonian

$$H = \sum_{\text{neighbors}} J(\tau(\sigma(\vec{x})), \tau(\sigma(\vec{x}')))(1 - \delta(\sigma(\vec{x}), \sigma(\vec{x}'))) + \lambda \sum_{\sigma} (a(\sigma) - A_{\text{target}}(\sigma))^2$$

without a component for the chemotaxis. When the model only depends on the Hamiltonian above, then it is the same as the CPM and will act that way. That means that the model will go to its most favorable energy state and that nothing more effects the model. Figure 38 shows the result when $\mu = 0$.

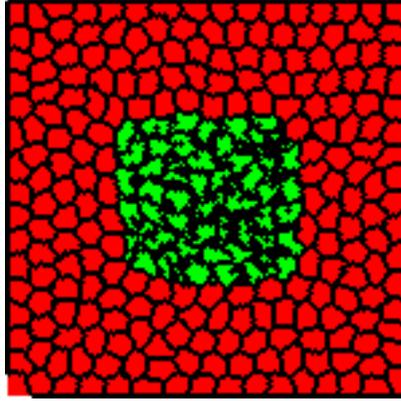


Figure 38: $\mu = 0$; Simulation state after 1250 MCS

Without any chemotaxis of the epithelial cells, all the cells will almost stay in their initial place. They are slightly moved into a position with a lower energy state, but nothing more. The most favorable energy state is that of a epithelial square inside the mesenchymal tissue, where the boundary energies and surface energies are at their lowest points.

Now set $\mu = \infty$, then $\Delta H_{\text{chemotaxis}} = -\infty$ when pixel \vec{x} has a lower concentration c_2 than pixel \vec{x}' in which \vec{x} tries to copy itself. On the other side is $\Delta H_{\text{chemotaxis}} = +\infty$ when \vec{x} tries to copy into \vec{x}' with an lower concentration c_2 . So chemotaxis gives a negative energy change of $\Delta H = -\infty$, where movements of epithelial cells away of the chemoattractant gives an energy change of $\Delta H = \infty$, causing into a probability of $e^{-\frac{\Delta H}{T}} = 0$. So the epithelial cells will only chemotact.

The mesenchymal cells will act the same as in the CPM, going to its most favorable energy state. Figure 39 shows the result of the EM model with $\mu = \infty$.

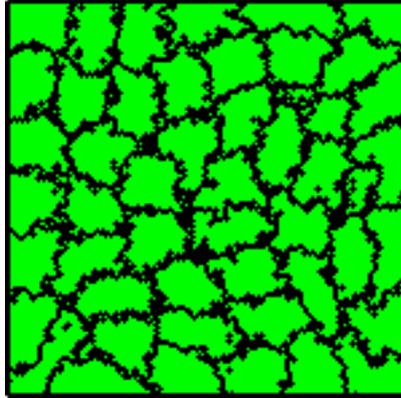


Figure 39: $\mu = \infty$; Simulation state after 1250 MCS and $\mu = 1,000,000$

In Figure 39 all of the mesenchymal cells are disappeared. The epithelial cells can only move by chemotaxis and each pixel with this cell type succeeds with probability 1 at a copy by chemotaxis. So the epithelial cells copy themselves into the mesenchymal cells, causing the last ones to disappear, because the probability that a copy of a pixel with $\tau = 2$ succeeds, depends on ΔH . The chemotaxis makes it possible for the epithelial cells to reach each mesenchymal cell, so at last only the epithelial cells are left.

After studying the extreme values of the temperature, a parameter sweep is done to find proper values where branches occur. The compactness is also measured and given in Figure 40.

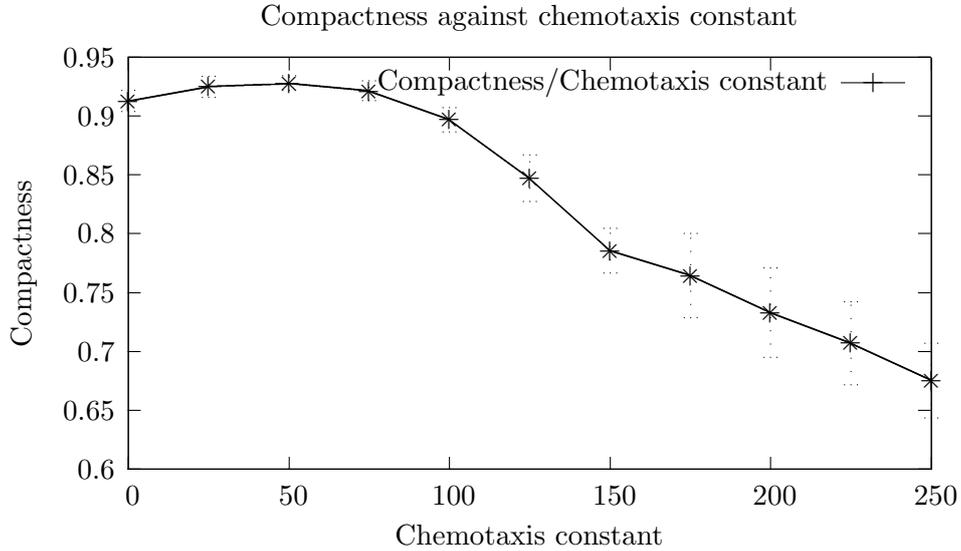


Figure 40: **Compactness against chemotaxis constant;** Mean and standard deviations from 10 simulations at each value

The graph in Figure 40 shows that the compactness decreases, when the value of the chemotaxis constant rises. Between $\mu = 0$ and $\mu = 50$ the compactness rises from $C = 0.913 \pm 0.0091$ to $C = 0.928 \pm 0.0089$. If $\mu = 0$ there is no chemotaxis of the epithelial cells to the mesenchymal cells and the model will thus go to its energetic most favorable state, the epithelial square inside the mesenchymal tissue. When the chemotaxis constant becomes $\mu = 25$ the chemotaxis is too weak to cause an effect. The ΔH in the direction of the chemotaxis becomes less than without chemotaxis and so copies that have a positive ΔH without chemotaxis can now have a negative ΔH , causing that the copy will succeed always. So the most favorable energy state will be when the epithelial cells at the boundary of the cluster will all have the same amount of mesenchymal neighbor cells, because then the chemotaxis is everywhere around the cluster the same and thus the energy cannot be lower. So there will be an almost perfect spheroid of epithelial cells and thus the compactness rises. When $\mu > 50$ the chemotaxis will be that strong that some epithelial cells move outwards the cluster, causing the compactness to drop.

At $\mu = 250$ the compactness is $C = 0.675$ with $sd = 0.0319$ and is still dropping, but the graph ends here because in the morphospace (Figure 41) the morphology at $\mu = 250$ shows that the cluster is fallen apart at some points. The chemotaxis is too strong to contain a cluster of epithelial cells.

Figure 41 shows the morphospace of the chemotaxis constant.

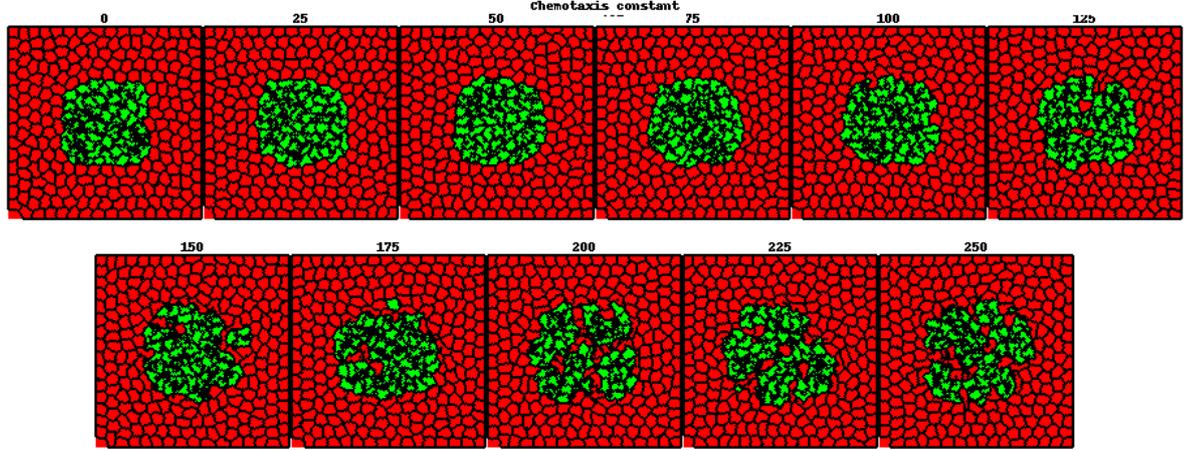


Figure 41: **Morphospace with different values of the chemotaxis constant;** Cell field after 1250 MCS

In Figure 41 the same behavior of the cells is found as in the graph in Figure 40. For $0 < \mu < 50$ the cluster forms a spheroid because of the weak chemotaxis, and for $\mu \geq 50$ the chemotaxis grows stronger and causes branches in the cluster. At $\mu = 250$ the chemotaxis is that strong that the cluster falls apart.

3.2.4 Conclusion

The EM model shows that branches occur when the parameters of the model are set as in Table 2. To find the best parameters of the model, the diffusion length, the temperature and the inhibition constant are further discussed. The diffusion length of c_1 at $L = 0$ gives that the model acts the same as the CPM and so the epithelial cluster will form a square inside the mesenchymal tissue. For $0 < L < 2.887$ the compactness rises from $C = 0.915$ to $C = 0.932$. For $2.887 < L < 8.660$ the compactness decreases to $C = 0.795$ and branches are found. At $L = \infty$ the epithelial cluster will fall apart due to chemotaxis. The diffusion length of c_2 at $L = 0$ gives that the model again works as the CPM and so a epithelial square will be formed. For $0 < L < 1.118$ the compactness decreases from $C = 0.876$ to $C = 0.524$. For $1.118 < L < 3.354$ the compactness rises again to $C = 0.747$ and branches are found. If $L = \infty$ the epithelial cells will form a spheroid. The temperature has only an effect at low temperatures. For $0 < T < 20$ the compactness drops steeply from $C = 0.881$ to $C = 0.776$ but remains the same at $T > 20$. The chemotaxis gives an epithelial spheroid for $0 < \mu < 50$ and for $\mu \geq 50$ the cluster shows branches until it falls apart at $\mu = 250$.

4 Discussion

The question that rises out of earlier experiments of Nelson et al. (2006) and Hirashima et al. (2009) was if epithelial tissue alone can form branching morphologies, or whether external signals initiate branching. In this article two different models were made, based on both experiments and other models.

The first model, the Inhibition Model, was based on the experiments of Nelson et al. (2006) and showed that with the right parameters chosen for the model, the autoinhibition of the epithelial cells suffices for branching morphogenesis (Figure 6).

The other model was the Epithelial Mesenchymal Model, based on the model given by Hirashima et al. (2009). They found that elastic epithelial tissue will move to fixed sources of grow factor driven by chemotaxis and thereby form branches in the cluster. The EMM showed that the non-elastic epithelial tissue will even form branches when the sources of grow factor are not fixed, but when the grow factors are produced by an autocrine loop between the epithelial cells and the mesenchymal tissue around them.

Branching morphogenesis occurs in both the IM and EMM and thus can be driven by autoinhibition or an autocrine loop with surrounding tissue. One strong difference between both models is the amount of Monte Carlo steps after when branching morphologies appear and disappear. For the EMM most branching morphologies are gone after 2500 MCS, against the existing of branching morphologies after 9000 MCS in the IM. The lack of elasticity in the EMM is one reason for this, compared to the results of Hirashima where branching morphologies last longer. According to the CPM the cells in both models are placed on a lattice and the dynamics are measured for each pixel on this lattice. Another way to simulate cell dynamics is to define the tissue as a fluid and cells as density. See Iber and Tanaka (2013) for this kind of models. Here there is no elasticity as in the model by Hirashima, but the tissue will be longer together than in the models discussed in this article. This because it is seen as a fluid instead of separated cells.

It is not known if branching morphogenesis prescribed by the discussed models is done the same way in nature and the precise parameter values are not experimentally known. Therefore it is important to do parameter studies, to see how the model behaves and to study the sensitivity of the model according to the parameters. When the model is more understood, similar changes can be done in the experiments to compare the results with the simulations.

Looking at Figure 12 (page 19) containing the morphospace with different diffusion constants, the model blows up at a diffusion constant of $D = 0.25$. The reason for this is that the model works with a numerical method to

compute the derivative of the concentration. This method, Euler forwards, takes big time steps, what can lead to a negative concentration in some steps. That is not possible, so the model blows up and then gives this figure. To investigate higher diffusion lengths it is necessary to take smaller time steps in order to prohibit the concentration to be negative or to lower the decay constant.

In Figure 14 (page 21), all the epithelial cells disappear and only the medium remains. This is a reaction of the model at higher temperatures. The cell motility is that high that the probability for a pixel to copy itself in its neighbor, causing a higher effective energy is also 1, because $e^{\frac{-\Delta H}{T}}$ becomes 1 when T moves to infinity. There is a lot more medium than epithelial cells, so all the pixels of type medium will copy themselves into the cells, causing the cells to disappear and once a cell is gone, it cannot come back.

The branches that occur in the EMM hold for about 1250 MCS and after that the cluster of epithelial cells falls apart. Further studies are necessary to conclude if this is a critical point in the model or that the epithelial cells needs more than only the autocrine loop to form branches and contain them for longer time.

Both the models give a way to form branches in a epithelial cluster and so produce the first steps of forming a branched organ, such as the lungs, kidneys or all kind of glands. A nice step for further studies is to see how changes in the adhesion energy effect both models. If the adhesion energy between epithelial cells increases, will the branches exist for longer time or will they never appear. Another nice step following this article is to see what happens when both the models are put together such that forming branches is driven by autoinhibition and an autocrine loop with the mesenchymal tissue at the same time. In that way a model can be made that comes closer to reality.

5 References

1. Nelson, C.M., VanDuijn, M.M., Inman, J.L., Fletcher, D.A., Bisseli, M.J. (2006) Tissue geometry determines sites of mammary branching morphogenesis in organotypic cultures. *Science*, 314, 298-300
2. Hirashima, T., Iwasa, Y., Morishita, Y. (2009) Dynamic modeling of branching morphogenesis of ureteric bud in early kidney development. *Journal of Theoretical Biology*, 259(1), 58-66
3. Merks, R., Newmand S., Glazier, J. (2004) Cell-oriented modeling of in vitro capillary development. In: Sloot P., Chopard, B., Hoekstra, A., eds. *Cellular Automata: 6th International Conference on Cellular Automata for Research and Industry*. Berlin: Springer Verlag, Lect. Notes Comput. Sc. 3305, 425-434
4. Gamba, A., Ambrosi, D., Coniglio, A., De Candia, A., Di Talia, S., et al. (2003) Percolation, morphogenesis, and Burgers dynamics in blood vessel formation. *Phys Rev Lett* 90
5. Merks, R., Perryn, E., Shirinifard, A., Glazier J. (2008) Contact-Inhibited Chemotaxis in De Novo and Sprouting Blood-Vessel Growth. *PLoS Comput Biol* 4(9)
6. Ambrosi, D., Gamba, A., Serini, G. (2004) Cell directional persistence and chemotaxis in vascular morphogenesis. *B Math Biol*, 66, 1851-1873
7. M. Swat, Gilberto L. Thomas, Julio M. Belmonte, A. Shirinifard, D.Hmeljak, J. A. Glazier (2012) Multi-Scale Modeling of Tissues Using CompuCell3D, *Computational Methods in Cell Biology, Methods in Cell Biology*, 110, 325-366
8. Palm, M., Merks, R. Large-scale parameter studies of cell-based models of tissue morphogenesis using CompuCell3D or VirtualLeaf
9. Tanaka, S., Iber, D. Inter-dependent tissue growth and turing patterning in a model for long bone development (2013) *Physical Biology*
10. Voss-Böhme, A. (2012) Multi-Scale Modeling in Morphogenesis: A Critical Analysis of the Cellular Potts Model *PLoS ONE* 7, 9

6 Appendix

6.1 XML files of IM and EMM

First the XML file of the IM will be given.

```
<CompuCell3D version="3.6.2">
  <Potts>
    <!-- Basic properties of CPM (GGH) algorithm -->
    <Dimensions x="150" y="150" z="1"/>
    <Steps>10000</Steps>
    <Temperature>150</Temperature>
    <NeighborOrder>3</NeighborOrder>
  </Potts>
  <Plugin Name="CellType">
    <!-- Listing all cell types in the simulation -->
    <CellType TypeId="0" TypeName="Medium"/>
    <CellType TypeId="1" TypeName="EC"/>
  </Plugin>
  <Plugin Name="Volume">
    <VolumeEnergyParameters CellType="EC" LambdaVolume="20.0"
    TargetType="EC"
  </Plugin>
  <Plugin Name="CenterOfMass">
    <!-- Module tracking center of mass of each cell -->
  </Plugin>
  <Plugin Name="Contact">
    <!-- Specification of adhesion energies -->
    <Energy Type1="Medium" Type2="Medium">0.0</Energy>
    <Energy Type1="Medium" Type2="EC">15</Energy>
    <Energy Type1="EC" Type2="EC">15</Energy>
    <NeighborOrder>4</NeighborOrder>
  </Plugin>
  <Plugin Name="Chemotaxis">
    <ChemicalField Name="c1"Source="FlexibleDiffusionSolverFE">
    <ChemotaxisByType ChemotactTowards="Medium" Lambda="150.0"
    Type="EC"
    </ChemicalField>
  </Plugin>
  <Plugin Name="Secretion">
    <!-- Specification of secretion properties of select cell
    types. -->
    <Field Name="c1">
      <Secretion Type="EC">0.013</Secretion>
    </Field>
  </Plugin>
  <Steppable Type="FlexibleDiffusionSolverFE">
    <!-- Specification of PDE solvers -->
```

```

<DiffusionField>
  <DiffusionData>
    <FieldName>c1</FieldName>
    <DiffusionConstant>0.24</DiffusionConstant>
    <DecayConstant>0.03</DecayConstant>
    <DoNotDecayIn>EC</DoNotDecayIn>
  </DiffusionData>
  <BoundaryConditions>
    <Plane Axis="X">
      <ConstantDerivative PlanePosition="Min" Value="0.0"/>
      <ConstantDerivative PlanePosition="Max" Value="0.0"/>
    </Plane>
    <Plane Axis="Y">
      <ConstantDerivative PlanePosition="Min" Value="0.0"/>
      <ConstantDerivative PlanePosition="Max" Value="0.0"/>
    </Plane>
    <Plane Axis="Z">
      <ConstantDerivative PlanePosition="Min" Value="0.0"/>
      <ConstantDerivative PlanePosition="Max" Value="0.0"/>
    </Plane>
  </BoundaryConditions>
</DiffusionField>
</Steppable>
<Steppable Type="PIFInitializer">
  <PIFName>rectangular.piff</PIFName>
</Steppable>
</CompuCell3D>

```

Now the XML file of the EMM will be given.

```
<CompuCell3D version="3.6.2">
  <Potts>
    <!-- Basic properties of CPM (GGH) algorithm -->
    <Dimensions x="150" y="150" z="1"/>
    <Steps>3000</Steps>
    <Temperature>20.0</Temperature>
    <NeighborOrder>3</NeighborOrder>
  </Potts>
  <Plugin Name="CellType">
    <!-- Listing all cell types in the simulation -->
    <CellType TypeId="0" TypeName="Medium"/>
    <CellType TypeId="1" TypeName="EC"/>
    <CellType TypeId="2" TypeName="Mesa"/>
  </Plugin>
  <Plugin Name="Volume">
    <VolumeEnergyParameters CellType="EC" LambdaVolume="20.0"
TargetVolume="74"/>
    <VolumeEnergyParameters CellType="Mesa" LambdaVolume="20.0"
TargetVolume="74"/>
  </Plugin>
  <Plugin Name="CenterOfMass">
    <!-- Module tracking center of mass of each cell -->
  </Plugin>
  <Plugin Name="Contact">
    <!-- Specification of adhesion energies -->
    <Energy Type1="Medium" Type2="Medium">0</Energy>
    <Energy Type1="Medium" Type2="EC">0</Energy>
    <Energy Type1="Medium" Type2="Mesa">0</Energy>
    <Energy Type1="EC" Type2="Mesa">8</Energy>
    <Energy Type1="EC" Type2="EC">1</Energy>
    <Energy Type1="Mesa" Type2="Mesa">10</Energy>
    <NeighborOrder>4</NeighborOrder>
  </Plugin>
  <Plugin Name="Chemotaxis">
    <!-- Specification of chemotaxis properties of select cell
types. -->
    <ChemicalField Name="c1" Source="FlexibleDiffusionSolverFE">
</ChemicalField>
    <ChemicalField Name="c2" Source="FlexibleDiffusionSolverFE">
      <ChemotaxisByType ChemotactTowards="Mesa" Lambda="150.0"
Type="EC"/>
    </ChemicalField>
```

```

</Plugin>
<Plugin Name="Secretion">
  <!-- Specification of secretion properties of select cell types. -->
  <Field Name="c1">
    <Secretion Type="EC">0.13</Secretion>
  </Field>
  <Field Name="c2">
    <Secretion Type="Mesa">0.00001</Secretion>
  </Field>
</Plugin>
<Steppable Type="FlexibleDiffusionSolverFE">
  <!-- Specification of PDE solvers -->
  <DiffusionField>
    <DiffusionData>
      <FieldName>c1</FieldName>
      <DiffusionConstant>0.24</DiffusionConstant>
      <DecayConstant>0.003</DecayConstant>
    </DiffusionData>
    <BoundaryConditions>
      <Plane Axis="X">
        <ConstantDerivative PlanePosition="Min" Value="0.0"/>
        <ConstantDerivative PlanePosition="Max" Value="0.0"/>
      </Plane>
      <Plane Axis="Y">
        <ConstantDerivative PlanePosition="Min" Value="0.0"/>
        <ConstantDerivative PlanePosition="Max" Value="0.0"/>
      </Plane>
    </BoundaryConditions>
  </DiffusionField>
  <DiffusionField>
    <DiffusionData>
      <FieldName>c2</FieldName>
      <DiffusionConstant>0.24</DiffusionConstant>
      <DecayConstant>0.02</DecayConstant>
      <DoNotDecayIn>Mesa</DoNotDecayIn>
    </DiffusionData>
    <BoundaryConditions>
      <Plane Axis="X">
        <ConstantDerivative PlanePosition="Min" Value="0.0"/>
        <ConstantDerivative PlanePosition="Max" Value="0.0"/>
      </Plane>
      <Plane Axis="Y">
        <ConstantDerivative PlanePosition="Min" Value="0.0"/>
        <ConstantDerivative PlanePosition="Max" Value="0.0"/>
      </Plane>
    </BoundaryConditions>
  </DiffusionField>

```

```

        </Plane>
        </BoundaryConditions>
    </DiffusionField>
</Steppable>
<Steppable Type="PIFInitializer">
    <PIFName>NewSimulation.piff</PIFName>
</Steppable>
</CompuCell3D>

```

6.2 Steppables

Here the steppable will be given used for the autocrine loop in the EMM.

```

from PySteppables import *
import CompuCell
import sys
class ECMesaSteppable(SteppableBasePy):

    def __init__(self, _simulator, _frequency=1):
        SteppableBasePy.__init__(self, _simulator, _frequency)
    def start(self):

    def step(self, mcs):
        field1=CompuCell.getConcentrationField(self.simulator, "c1")
        #veld van c1
        field2=CompuCell.getConcentrationField(self.simulator, "c2")
        #veld van c2
        for cell in self.cellListByType(2):
            x=int(cell.xCOM)           #x-waarde van cel
            y=int(cell.yCOM)           #y-waarde van cel
            field1_cell=field1[x,y,0]  #conc c1 bij cel
            field2_cell=field2[x,y,0]  #conc c2 bij cel
            if field1_cell >= 1:       #als conc c1 >= 1 dan
                field2_cell=field2_cell+0.2*field1_cell
                #verhoog conc c2 met helft conc c1
                field2[x,y,0]=field2_cell
    def finish(self):
        # Finish Function gets called after the last MCS
        pass

```