Spatial separation of bistable cell types in heterogeneous populations

H. Lindhorst ∗ M. Hussain ∗ S. Waldherr ∗

Abstract: Cellular differentiation is commonly modeled by bistable switches under the influence of a diffusive morphogen. In higher organisms, differentiation is relevant for the formation of different tissue types, which require a clear spatial separation. Cellular heterogeneity in the sensitivity to the morphogen impairs the formation of a clear boundary. We are investigating the role of intercellular communication and synchronized switching for tissue separation. We construct a spatially distributed model that combines a cellular population of bistable switches with the diffusion of a morphogen in the extracellular medium. The model is able to predict a clear separation in different tissue types despite cellular heterogeneity. Based on that model, we formulate an objective function that measures the quality of the spatial separation, and use numerical optimization to determine parameter values that yield a clear tissue boundary.

Keywords: Population dynamics, Bistable switch, Distributed systems, Synchronized switching

1. INTRODUCTION

An important part in understanding the process of morphogenesis is the concept of cell differentiation. The basic concept of morphogenesis is being investigated since the early 1950s (Turing, 1952) and the role of morphogens in the formation of tissue is still intensively researched. Especially in the area of tissue engineering the application of morphogen is used to change e.g. stem cells to the desired cell type (Kishida et al., 2008). Usually, the concept of cell differentiation is modeled with a multi-stable differential equation for single cells (Chickarmane et al., 2009; Huang et al., 2007; Schittler et al., 2010). In this sense each stable state corresponds to a specific cell type. We sometimes refer to multi-stable systems in biology as biological switches as they are not only used to model cell differentiation but are also used to analyze changes in the internal dynamics without a real change of the cell type. A typical example is the lactose utilization in E. coli. (Özbudak et al., 2004), where the internal metabolism is controlled by external glucose and thio-methylgalactoside molecules. General signaling networks and their ability to reach bistability are analyzed in (Essig et al., 2007). Usually, the analysis of bistable multicellular systems is strongly connected to the idea of consensus in a multi-agent system (Schmidt et al., 2010),(Ernst et al., 1998) or the synchronization of coupled biological clocks (Winfree, 2001).

In the literature one can find different models for the coupling of the cells. (Schmidt et al., 2010) presents an algebraic connection based on a fixed graph structure.

While this approach gives insights in the structure of the consensus space, we are rather interested in the idea of diffusive coupling via the medium. This model is better suited for typical cell-to-cell communication (Fuqua et al., 2001) as the interaction graph can usually not be predicted. In previous work, it has been shown that fast diffusive exchange between cells and the medium leads to bistability on the population level and thus synchronized switching among cells (Waldherr and Allgöwer, 2012). However, the singular perturbation method requires a sufficient fast diffusive process and does not consider any information about the spatial arrangement of the cells.

In this work, we are investigating how the diffusive process in the medium interacts with the exchange between cells and medium. Thereby, we locate the cells on a one-dimensional space and use a cell model, which can differentiate between two cell types under the influence of a morphogen. While all cells are mechanistically of the same type, we include a heterogeneity in the population by making the switching conditions dependent on a stochastically distributed parameter (Mantzaris, 2007). This enables us to not only predict a synchronized behavior, but our model allows the population to diversify in two spatially separated regions where the cells are synchronized. We emphasize on this separation and use optimization methods to check under which conditions a clear-cut separation occurs. This may find application in tissue engineering, when more complex structure consisting of more than one cell type are needed.

The paper is structured as follows. In Section 2 we present the model for internal dynamics and the external concentration. Furthermore, the exchange between these concentrations is explained. In Section 3 a discretization for the spatial dimension is constructed. This means we transform
the arising partial differential equation into a system of coupled differential equations. To check whether the model can predict the wanted separation we draw up a parameter optimization problem in Section 4. Finally, in Section 5 we apply this optimization to a numerical example and show for which parameter range a separation can occur.

2. MODELING THE CELL POPULATION

Consider a heterogeneous cell population of \( N \) single cells. The intracellular dynamics for the morphogen \( x \in \mathbb{R}_+ \) are represented by the non-linear differential equation

\[
\dot{x}_i = \frac{k_i x_i^2}{1 + x_i^2} - x_i + u, \quad i = 1, \ldots, N,
\]

where \( x \in \mathbb{R}_+ \) is given as a concentration, \( u \in \mathbb{R}_+ \) is an external input, and \( k_i \in (2, \infty) \) is a stochastic parameter. The input \( u \) is assumed to act directly on the cells and does not interact with the medium. For example, this input can be a locally fixed chemical prompting the cell to produce the morphogen. If \( u \) is set to zero, the internal dynamics have three equilibrium points \( \bar{x}_i \) dependent on the stochastic parameter \( k_i \):

\[
\bar{x}_{i,a} = \frac{k_i}{2} + \sqrt{\frac{k_i^2}{4} - 1}, \quad \bar{x}_{i,u} = \frac{k_i}{2} - \sqrt{\frac{k_i^2}{4} - 1},
\]

where \( \bar{x}_{i,a}(k_i) \) are two stable equilibria and \( \bar{x}_{i,u}(k_i) \) is unstable. The actual value of the non-zero stable equilibrium may vary for each cell yet it represents the same operating state for the cells. Therefore, the whole population can be perceived as bistable. We refer to the equilibrium at \( x = 0 \) as the off-state and the non-zero one as the on-state. To understand the behavior of the cells under the influence of the input, we do a bifurcation analysis with respect to \( u \) as shown in Figure 1. There are two saddle-node bifurcations occurring at the critical values

\[
u_{1/2}^* = \frac{5k_i + 2\sqrt{k_i^2 - 3} \pm \sqrt{k_i^4 - 3 - k_i^2}}{6(k_i^2 + 1)}.
\]

This means for bigger values of \( k_i \) the critical value of the lower bifurcation tends to zero

\[
\lim_{k_i \to \infty} \nu_{1/2}^* = 0.
\]

Therefore, cells with large values of \( k_i \) tend to activate, even under small disturbances in the off-state. This makes the strength of the input \( u \) very important. In the following work, we assume \( u \) is only applied as a pulse-like stimulus in the form

\[
u(p, t) = \begin{cases} -m p + u_{\max}, & t \leq t_{\text{off}}, \\ 0, & \text{else}, \end{cases}
\]

with \( p \) representing the position. By these means the internal dynamics overtake for \( t > t_{\text{off}} \).

2.1 Spatial distribution of cells

The cells are distributed on a one-dimensional space with length \( l \), with the position denoted as \( p \in [0, l] \). We can address the cells and the internal morphogen concentration \( x_i \) by the location of the cell center \( t_i \in [0, l] \). Depending on the length \( l \) and the stochastic distribution of \( k_i \), we trim \( u_{\max} \) and \( m \in \mathbb{R}_+ \) (cf. (2)) so that two-thirds of the population will receive an input greater than the critical value \( u_i^*(K) \) for the expected value \( K \) of \( k_i \). One would expect this to be sufficient for a clear separation of the cell types, but numerical trials have shown, that this is usually not the case, due to the stochastic heterogeneity in \( k_i \). Typical results of a simulation with this setup are depicted in Figure 2. For this simulation we used a population of \( N = 100 \) cells with equal initial values \( x_i^0 = 0.2, \ i = 1, \ldots, 100 \). For the distributed parameter we assume a gamma distributed parameter \( \tilde{k} \) has a density function

\[
\mathbb{P}(\tilde{k}) = k^{s-1} e^{-k/\theta} / \Gamma(s),
\]

with shape \( s = 0.3 \), scale \( \theta = 1 \) and the Gamma function \( \Gamma \). The expected value of this distribution is \( \tilde{K} = 0.3 \) and we define the cell specific parameters as

\[
k_i = 2.7 + \hat{k}.
\]

Then \( k_i \) is also gamma distributed with expected value \( K = 3 \). The separation in Fig. 2 should take place around position \( p = 61 \), but there are still four cells on the right which did switch to the on-state. Thus, we can not really speak of a clear separation as the cells between \( p = 61 \) and \( p = 78 \) are in different states. In the context of cell differentiation this means no clear boundary between the tissue types can form. This behavior always occurs, if some \( k_i \) values are large for cells far on the right.

To tackle this problem we introduce a coupling of the cells via the medium as suggested in (Waldherr and Allgöwer, 2012). However, instead of assuming a well mixed medium at all times we now take the spatial distribution of the cells and the diffusion of the morphogen in the medium into account.
Fig. 2. System wide equilibrium for internal concentrations $x_i$ are plotted over the position. Input is chosen as in (2) and initial values is $x_i^0 = 0.2$ for all cells.

2.2 Diffusive coupling

For the coupling between the cells we look at the concentration of the morphogen in the medium $x_i(p,t), p \in [0,\ell]$ and refer to it as the external concentration. We assume the morphogen is only produced inside the cells. The cells are modelled with a circular shape and individual volume $V_{C,i}$. We introduce cell specific support functions $h_i(p) \in \mathbb{R}$ containing the location and the diameter of each cell. For more detail on this function see Appendix A. In cooperation with the coupling strength constant $k_C$ the exchange flux between a cell and the medium can be expressed by integration over the length of the system.

In the medium the morphogen diffuses according to Fick’s law with the diffusion constant $k_D$. Finally, the morphogen degenerates outside the cells with a rate constant $k_e$. Putting this together we can describe the dynamics of the outside concentration as the partial differential equation

$$\frac{dx_e}{dt} (p,t) = k_D \frac{\partial^2 x_e}{\partial p^2} (p,t) - k_e x_e (p,t) + \sum_{i=1}^{N} \frac{k_C}{V_{C,i}} h_i(p) (x_i(t) - x_e(p,t)).$$

(5)

Accordingly, we extend the equations (1) for all cells as

$$\frac{dx_i}{dt} = k_i x_i^2 (1+x_i^2) - x_i + u - \int_{-\infty}^{\infty} \frac{k_C}{V_{C,i}} h_i(p) (x_e(p) - x_i)dp.$$  

(6)

Of course, the next step to check whether this coupling can produce the desired separation is by implementing the system and run simulations.

3. IMPLEMENTATION

For the numerical analysis of the PDE system (5), (6) a discretization of the spatial dimension is necessary. We want to utilize a finite element method and constrain the diffusion between those elements. To do this, the length $l$ of the system is divided into $M$ equally-sized compartments. The size of the compartments is so small that each compartment can be assumed as well mixed. Therefore, we assign each compartment an external concentration $x_{e,m}$, $m \in \{1,\ldots,M\}$. Depending on the positions of the cells, a different number of the cells can be present in each compartment. We capture this by counting the cells in compartment 1 as $m_1$. The cells in compartment 1 and 2 are counted together as $m_{21}$. $m_i$ is the sum of the numbers of cells in all compartments up to the $i$-th compartment. Therefore, $m_M$ is equal to the total number of cells $N$. Assuming only neighbouring compartments can interact, the diffusion between compartments $j$ to $j+1$, $j \neq \{1, M\}$ is described as

$$v_D(j, j+1) = k_D (x_{e,j} - x_{e,j+1}).$$

We can then write down the discretization of the original PDE (5) as a collection of coupled differential equations

$$\frac{dx_{e,1}}{dt} = -k_e x_{e,1} + k_D (-x_{e,1} + x_{e,2}) + \frac{k_C}{V_e} \sum_{i=1}^{m_1} (x_{e,2} - x_i)$$

(7a)

$$\frac{dx_{e,j}}{dt} = -k_e x_{e,j} + k_D (x_{e,j-1} - 2x_{e,j} + x_{e,j+1}) + \frac{k_C}{V_e} \sum_{i=m_{j-1}+1}^{m_j} (x_{e,i} - x_{e,j}), j \in \{2,\ldots,M-1\}$$

(7b)

$$\frac{dx_{e,M}}{dt} = -k_e x_{e,M} + k_D (x_{e,M-1} - x_{e,M}) + \frac{k_C}{V_e} \sum_{i=m_{M-1}+1}^{N} (x_{e,M} - x_i).$$

(7c)

The exchange has to be weighted by the volume of the compartments $V_e$ to ensure the interaction between the two concentration variables is balanced. We assume here implicitly, that the cells are contained completely in the compartments. This assumption enables us to leave out the support function (A.1) in the discretization. The equations for the cells are adjusted to the discretization as

$$\frac{dx_i}{dt} = \frac{k_i x_i^2}{1+x_i^2} - x_i + u_i(t) - \frac{k_C}{V_{C,i}} (x_{e,j} - x_i).$$

(8)

The positions of the cells define the compartment they are interacting with. Hence, for $i \in \{m_{j-1}+1, m_j\}$ the exchange happens between the cell $x_i$ and the external concentration $x_{e,j}$.

For arbitrary chosen $k_D, k_C, k_e$ and a single compartment for each cell the system (7),(8) tends to put the whole population to a consensus. The morphogen produced by the cells in the on-state diffuses to the right and activates the cells one by one. This effect can be seen in Figure 3. For this example we use $k_e = 0.3$, $k_D = 5$, $k_c = 1$, gamma distributed $k_i$ with expected value $K = 3$, an initial value $x_i^0 = 0.2, \forall i$, and assume there is initially no external morphogen. We use the initial stimulus(2) for $t < 5$.

This effect of consensus usually occurs, when the diffusion coefficient $k_D$ is large while the degeneration constant $k_e$ is small. These results were already discussed in (Waldherr and Allgöwer, 2012) under the assumption of a well-mixed conditions.
extracellular medium, i.e. a very large diffusion constant $k_D$. For large values of $k_e$ the other consensus option usually occurs and the whole population quickly tends to the off-state.

However, by raising the number of cells for each compartment to three and making a lucky guess for the parameters we got the results depicted in Figure 5. Here we can see a fuzzy separation happening. But it is still far from being a nice clear cut between the operating states. Thus, we introduce an optimization problem to check whether our model can produce the intended clear cut.

4. PARAMETER OPTIMIZATION

For the parameter optimization we assume the degradation rate $k_e$ to be fixed. Thus, we only look for value pairs $k_C, k_D$, which lead to a clear spatial separation of the population. These values are obtained by standard tools from mathematical optimization. First, we construct an objective function measuring the quality of the separation between the cell types. We are only interested in the population wide equilibrium of the cells, so we write in short $y(x^0, x^1_C, k_C, k_D) = (y_1, \ldots, y_N)$ for these equilibria. They depend on the chosen initial values and parameters. We suggest an objective function as

$$J(y) = \sum_{i=1}^{N-1} |\arctan(5(y_{i+1}) - \arctan(5(y_{i+1} - 1))|$$

$$\quad + (y_1 - \bar{y})^4 + (y_N)^4,$$

with $\bar{y}$ describing the on-state for the expected value of $k_i$.

The objective function consists of three distinct parts. The sum counts the difference between adjacent cells. While the inner function $\arctan(5(y_i - 1))$ is chosen in a way to put a huge penalty on adjacent cells in different operating

states, e.g. $y_i$ is in the off- and $y_{i+1}$ is in the on-state. At the same time adjacent cells in the on-state do not affect the value of the objective function as heterogeneity effects are smoothed out. To ensure the left part of the population is active and the right part is in the off-state, we put additional weight on the first cell and the last cell. This is necessary as the objective function would otherwise reach its minimum at a population with $y_i = 0, \forall i$. For given values $x^0, x^1_C, k_e$ the continuous optimization problem is defined as

$$\min_{k_C,k_D} J(y),$$

with respect to

$y$ is a population wide equilibrium of (5),(6)

$k_C, k_D \geq 0$.

For the implementation the objective function is discretized as

$$J(\bar{y}) = \sum_{j=1}^{M-1} |\arctan(5(\bar{y}_j - 1)) - \arctan(5(\bar{y}_{j+1} - 1))|$$

$$\quad + (\bar{y}_1 - \bar{y})^4 + (\bar{y}_N)^4,$$

where $\bar{y}_j$ is the mean concentration of all cells in compartment $j$ at the population wide equilibrium for the differential equation system (7a),(7b),(7c) and (8).

5. NUMERICAL EXAMPLE

In the numerical example we assume all cells keep a constant volume of $V_C = 1$ with radius $r_i = 1$. The population consists of $N = 300$ cells. They are synchronized and start all at the same internal morphogen concentration $x_i(t=0) = 0.2, i = 1, \ldots, 300$. As previously, we use gamma distributed parameters $k_i \in (2, \infty)$ defined by equations (3),(4). So the expected value is $K = 3$. We split the spatial dimension into $M = 100$ pieces of equal volume $V_C = 4$. Assuming the cells are evenly distributed along the spatial dimension, each compartment contains exactly three cells. The values for $k_i$ are fixed for the following simulations to keep the results comparable. We further assume there is no morphogen present in the medium at the initial time $t = 0$. As previously, we use the ramp function $u(p, t)$ we use the ramp function as explained in (2). We choose the determining values as $m = 6.25 \cdot 10^{-4}$, $u_{\text{max}} = 0.25$, and $t_{\text{off}} = 15$. With this ramp input we expect that roughly two thirds of the population will activate and go to the on-state, cf. Figure 2. As previously discussed, we fix the value for outside degradation at $k_e = 0.5$ and want to apply the discrete variant of the minimization approach. The numerical implementation of the ODE system (7a-c) and (8) is done in Python. The solver we use is part of the scipy-package\(^1\) and is a method based on backward differentiation formulas for stiff problems on a fixed time grid. For the step-size we chose $h = 0.01$. The simulation is run until a system wide equilibrium $y$ is reached. Figure 4 shows the values of the objective function for different values of $k_C, k_D$. The values are plotted on the grid $[0.3 + n \cdot 0.05] \mid n = 1, \ldots, 40]$. Small values for the objective function (depicted in blue) are the desired results. As can

\(^1\) http://www.scipy.org/
An issue with our approach lies in the fact, that we have two states for our cells but only one morphogen. We be seen in the plot, for parameter values $k_C \in [0.35, 0.6]$ and $k_D \in [0.3, 1.7]$ the results are nearly indistinguishable. However, it is to be expected, that very small changes of the parameter values lead to very different results in terms of the separation.

For example we pick the value set $k_C = 0.5$, $k_D = 0.8$ and show the equilibrium concentrations in Figure 5. We can see the results look very similar to the results we presented in Figure 2 without interaction of the individual cells. However, we use these values as initial guess for the minimization problem and apply a L-BFGS-B algorithm (Byrd et al., 1995), which is part of the scipy.minimization toolbox in Python. The result is plotted in Figure 6 for the locally optimal parameter set $k_C = 0.403526$, $k_D = 0.81259$. While this result most likely is no global optimum, it already achieves the desired result for our task. The separation takes place at position $p = 52$ and we have no cells in the “wrong” state on either side of it. So even if there would be a global minimum in terms of the objective function, from the original objective the result is as good as it can get.

6. CONCLUSION

Based on a bistable model of cell differentiation, we showed that cellular heterogeneity will impair the formation of a clear tissue separation under a common spatially distributed differentiation stimulus. To reconcile this issue, we have developed a mathematical model for a population of bistable cellular switches which are coupled via chemical diffusion in the extracellular medium. By coupling the cells via the medium, we can achieve a compensation for the heterogeneity in the cells’ sensitivity to the differentiation stimulus. Depending on the values of diffusion parameters, this model may give rise to an overall synchronization of the switch states, or lead to a spatial separation of the two states. With a suitably constructed objective function, we can determine suitable values of diffusion parameters $k_c$ and $k_D$ to achieve a clear tissue separation. However, our analysis also showed that small changes in these parameters may already impair the tissue separation. It is thus likely that additional mechanisms are present in living systems to ensure further robustness.

An issue with our approach lies in the fact, that we have two states for our cells but only one morphogen. We
expect our method for the separation to get more robust, if we include a second morphogen, which prompts the cells to go to back to the off-state (Wittmann et al., 2009). This idea can then be generalized to multi-stable systems, where each stable operating point is coupled to a specific morphogen (or a combination of those) (Schittler et al., 2010).

Another approach to improve our method, is by allowing the diffusion rates to change over time or space. This clearly enables one to control the differentiation process much better, although this might prove more challenging in experimental application.

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Appendix A. SUPPORT FUNCTION

The support function $h_i$ used in the model, are in fact hat functions covering the length of the cells, see Figure A.1. For circular cells with position $l_i$ and radius $r_i$, the support functions are defined as

$$h_i(p) = \begin{cases} 
\frac{1}{r_i^2} (p + \frac{1}{r_i} - l_i - r_i) & l_i - r_i \leq p \leq l_i \\
\frac{-1}{r_i^2} (p + \frac{1}{r_i} - l_i) & l_i \leq p \leq l_i + r_i \\
0 & \text{else}.
\end{cases}$$

(A.1)

Please note $\int_{-\infty}^{\infty} h_i(p) \, dp = 1$. In this context the support function do not simply specify the location of the cells, but rather put a weight on the exchange flux between cell and medium. Thus, the flux is strongest over the middle of the cell and weakens to the cell boundary. Therefore, the total diffusive exchange flux between cell $i$ and the medium is simply obtained by integrating over the spatially distributed flux.

The reason to use these support functions is to avoid single point sources or sinks as those would result in singularities of the PDE. This can lead to a variety of problems in solving the PDE, especially if the cells are very near to each other. While in our discretization the support functions no longer occur, we are confident their inclusion may be beneficial for other discretization techniques.

Fig. A.1. Graphic presentation of the support function $h_i$. 