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A Stochastic Broadcast Feedback Approach to Regulating Cell Population Morphology for Microfluidic Angiogenesis Platforms

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Abstract—This paper presents a framework for controlling the development of a vascular system in an in vitro angiogenesis process. Based on online measurement of cell growth and a stochastic cell population model, a closed-loop control system is developed for regulating the process of cell migration and vascular system development. Angiogenesis is considered in a microfluidic environment, where chemical and mechanical stimuli can be applied to the cell population. A systems-level description of the angiogenesis process is formulated, and a control scheme that chooses an optimal sequence of control inputs to drive collective cell patterns toward a desired goal is presented in this paper. In response to control inputs, the k-step ahead prediction of morphologic pattern measures is evaluated, and the input that minimizes expected squared error between the future measure and its desired value is selected for the current control. Initial simulation experiments demonstrate that vascular development can be guided toward a desired morphologic pattern using this technique.

Index Terms—Angiogenesis, biological cells, biological control systems, biological systems, microfluidic devices, population control, stochastic processes, vascular development.

I. INTRODUCTION

ANGIOGENESIS is the process of growing or extending a vascular network into a tissue matrix from a preexisting vascular system. Understanding its mechanism and regulating the growth process are critically important in many research areas, ranging from cancer treatment and wound healing to morphogenesis, stem cells, and tissue engineering. During angiogenesis, endothelial cells (ECs) sprout from existing vasculature and extend into the surrounding tissue. In response to local chemical and mechanical stimuli, each cell takes a series of spontaneous actions; it may migrate, proliferate, stay, die, etc. As a result, a population of cells creates a new vascular network. As a result, a population of cells creates a new vascular network. The population morphology is selected for the current control. Initial simulation experiments demonstrate that vascular development can be guided toward a desired morphologic pattern using this technique.

In vitro angiogenesis process by actively modulating chemical and mechanical stimuli is a truly challenging research issue, which will have a significant impact on broad biological engineering and medical fields. This paper addresses control of the angiogenesis process in an in vitro microfluidic environment. Fig. 1 illustrates an in vitro microfluidic flow chamber providing a proper environment for the cells to grow. Various angiogenic growth factors, such as vascular endothelial growth factor (VEGF), as well as inhibitory factors are supplied through microfluidic channels. Physical factors affecting the cell behaviors, such as interstitial flow rate, pressure, and temperature, can also be controlled in the chamber [1]. This microfluidic apparatus can not only create a desirable in vitro environment for the cells, but also facilitates monitoring growth by microscopy or other means, and process intervention by manipulating the chemical and physical factors.

This microfluidic technology opens up the possibility of actively controlling the cell growth process. However, angiogenesis and cell population growth processes differ in many ways from traditional engineered systems, where control technology has been applied successfully. First, the system consists of a population of cells, each having an independent local controller to perform a specific class of functions. Collective behavior of the cells exhibits meaningful functionality, such as constructing a vascular network. Second, cells live in a “wet” environment, where signals propagate through diffusion. Stimuli to the process affect broad regions of the cells. It is infeasible to directly control the behavior of each and every cell in the population. Available inputs are broadcast in nature, influencing the multitude of cells. Further, it is not necessary, or even desirable, to control each and every cell. In generating a vascular network, the system as a whole should satisfy certain collective requirements, such as vascular density in the matrix, rate of branching, rate of growth, etc. Rather than the behavior of each individual.
cell, correct development of the cellular population as a whole is important in developing a useful vascular system. The authors’ group is currently developing a detailed stochastic model of cell behavior in the wet environment [2]. The focus of this paper is on the development of a control framework for regulating the morphology of cell population growth. We will pose a systems-level description of angiogenesis as a process producing collective outputs in response to a set of input stimuli that we can manipulate. These stimuli propagate through the wet environment and reach individual cells as “broadcast” signals. This creates a unique control problem where collective behaviors of individual cells must be controlled based on broadcast inputs that pervasively influence a population of cells, rather than driving each cell individually. In the following, a systems-level description of the angiogenesis process will be obtained from the literature, and a broadcast control method for generating a sequence of broadcast inputs that will drive expected collective cell patterns toward a desired goal will be developed. Simulation experiments based on both the microfluidic bioreactor and a stochastic model being developed in the authors’ group [2] will demonstrate that the collective cell distribution can be guided toward a desirable morphologic pattern with the proposed broadcast feedback control.

II. ANGIogenESIS Process

A. In Vitro Microfluidic Station

Microfluidics technology provides us with an apparatus that facilitates feedback control of an angiogenic growth process. Here, we are mainly interested in the development of endothelial cell sprouts in in vitro experimentation, as shown in Fig. 1. In these devices, collagen gel is placed between two flow channels. Through one channel, ECs are seeded on the side of the channel coinciding with the collagen gel. Through the other channel, angiogenic growth factors (AGFs) such as VEGF are supplied. The pressure across the gel scaffold and the temperature are controllable. After an AGF is supplied at one end of the gel scaffold, the AGF diffuses through the gel, which is generically referred to as an extracellular matrix. The AGF binds with receptors in the ECs, driving the cells to proliferate and migrate [3]. After the ECs are triggered by the AGF, some cells differentiate into tip cells, which drive into the matrix in the direction of increasing AGF concentration. Other cells tend to follow along behind the tip cells (see Fig. 2). Some cells along a sprout branch may decide to differentiate into tip cells themselves and generate a new branch.

We are able to observe the development of the vascular system in the microfluidic device via phase contrast microscopy. Fig. 3 shows an image of one such microfluidic experiment in between two polydimethylsiloxane (PDMS) supports. Cells are seeded at the bottom through the microfluidic channel, and sprouts are being developed from the seeded cells.

B. Systems-Level Description of Angiogenesis

Angiogenesis is extremely complex and has not been fully understood yet, despite numerous experimental data and theoretical analyses over the past 40 years [3]–[9]. Reviewing the vast angiogenesis literature from the system dynamics and control viewpoint, the key components for controlling the angiogenesis process can be summarized as follows.

1) Matrix field model: Angiogenesis takes place in a matrix structure called the “matrix field,” where various chemicals (AGF, etc.) are diffused, and pressure, temperature and flow are distributed over a 3-D (or 2-D) space [3]. Each point in the matrix field has state variables representing the concentration of each chemical as well as pressure, flow rate, temperature, and others.

2) Cell behavior model: Being exposed to the matrix field, each cell receives chemical and mechanical “cues” from its surroundings, and makes a decision whether to migrate, divide, stay, or die, and in case of migration, which way to move. The details of cue-signal-response mechanisms are highly complex, but the cell’s behavior can be modeled as a stochastic agent that takes a series of spontaneous actions.

As a collective outcome of each cell’s decision, the multitude of cells exhibits a morphological pattern, leading to a vascular network formation. Incorporating the matrix field and the cell behavior model into the morphological formation process, we
can obtain a system-level description of angiogenesis, as shown in Fig. 4, for which we will develop a control method.

III. CONTROL FRAMEWORK

A. Control Problem Statement

The objective of the feedback control is to drive the morphological formation in a desired direction. To this end, we first define metrics of morphologic patterns, i.e., the output variables that we want to regulate. These include density and length of EC strings and other features relevant to the formation of functional patterns. In general, let $Y_t = [y_t^1, y_t^2, \ldots, y_t^n]^T$ be a collective representation of morphologic outputs at time $t$. Morphologic outputs $Y_t$ are determined by the cell distribution, i.e., the collection of coordinates of individual cells $Z_t$. It can be written as follows:

$$Y_t = h(Z_t, t).$$  

(1)

In Fig. 4, $Z_t$ is called the cell "constellation." To be useful for control, the cell constellation $Z_t$ should be observable via available instrumentation, such as imaging.

As shown in the block diagram in Fig. 4, the morphologic outputs $Y_t$ are compared to their desired values $Y_d = [y_d^1, y_d^2, \ldots, y_d^n]^T$, and the control $U_t$ is determined so that the discrepancy between $Y_t$ and $Y_d$ may be reduced. Note that the input is a vector of broadcast input variables $U_t = [u_t^1, u_t^2, \ldots, u_t^n]^T$ that can be manipulated in the microfluidic in vitro platform described before. These include temperature, temperature gradient, flow rate, pressure, various AGP partial pressures, etc. As $U_t$ is given, its effect propagates through the matrix field, varying the state of each point in the field. Let $X_t$ be the collective expression of the state of each point in the matrix field. The state transition of the matrix field may be governed by diffusion and microfluid channel dynamics, and can be written as a state-transition equation

$$X_{t+1} = f(U_t, X_t, Z_t, t) + \omega_t$$  

(2)

where $\omega_t$ is the process noise. Note that the previous equation includes constellation $Z_t$ because the existence of cells may alter the diffusion dynamics as well as the matrix properties.

Based on existing models, cells are treated as discrete agents that live in a grid world and respond, often probabilistically, to the field state $X_t$ [3]–[8]. The authors’ group is currently developing a model where each cell is treated as a random decision maker that can migrate, proliferate, stay, or die [2]. In most models, branch lengthening is assumed to be dominated by a biased random walk of the cell at the tip of the branch [4], [6]. Branch lengthening is often handled by assuming a probability per unit length of a new branch forming from an existing branch, as in [4]. The following description can be applied to any of the previously mentioned models. Let $\pi_{ij}$ be the probability of state transition from a current state $S_i$ to a new state $S_j$, collectively represented in matrix form as

$$P_t = \{\pi_{ij}(X_t)\}.$$  

(3)

Note that state-transition probabilities $P_t$ are modulated by field state $X_t$, which is controlled by the broadcast input $U_t$. With this probability matrix, each agent changes its location in the matrix field. As a result, the constellation $Z_t$ is updated to $Z_{t+1}$ as

$$Z_{t+1} = g(P_t, Z_t).$$  

(4)

B. Stochastic Optimal Control

Now the question is how to generate the input $U_t$, so that the current output $Y_t$ may be driven toward its desired value $Y_d$. Unlike traditional feedback control, broadcast inputs $U_t$ merely affect the cell’s stochastic behavior by modulating their state-transition probabilities $P_t$. The broadcast inputs are pervasive and no individual cells are directly controlled to desired locations. However, a desirable input can be obtained as long as the response of the cell population to various inputs is predictable.

Let the random variable $Y_{t+1}$ describe the morphologic output at time $t+1$ based on the model given by (1)–(4) with current field state $X_t$, constellation $Z_t$, and broadcast input $U_t$: $\Pr\{Y_{t+1}\} = \Pr\{Y_{t+1}(U_t; Z_t, X_t)\}$. A primitive way of determining the broadcast input $U_t$ is to find the one that minimizes the mean-squared norm of discrepancy between $Y_{t+1}$ and $Y_d$

$$U_t^* = \arg\min_{U_t \in D_U} E\left[|Y_d - \hat{Y}_{t+1}(U_t; X_t, Z_t)|^2\right]$$  

(5)

where $D_U$ is a set of all admissible broadcast inputs. The mean-squared error can be computed through numerical simulations, e.g., Monte Carlo simulation of the model. Considering the complexity of the cell growth process, which includes a long sequence of stochastic decisions by the multitude of cells, the one-step-ahead prediction evaluated in (5) cannot always provide a sound basis for evaluating morphological performance of the cell growth. Rather, multiple steps of transitions should be considered to elaborate whether the morphologic outcome is getting closer to the desired goal. Let $Y_{t+k}$ be the random variable describing the morphologic output at time $t+k$, given a sequence of broadcast inputs $U_t, \ldots, U_{t+k-1}$. We determine the optimal input for time $t$, $U_t^*$, to be the first input of a sequence that minimizes the mean squared error at time $t+k$ as

$$\min_{U_t, \ldots, U_{t+k-1} \in D_U} E\left[|Y_d - \hat{Y}_{t+k}(U_t, \ldots, U_{t+k-1})|^2\right]$$

$$= \min_{U_t, \ldots, U_{t+k-1} \in D_U} \left\{E\left[|\hat{Y}_{t+k}(U_t, \ldots, U_{t+k-1}) - Y_d|^2\right] + \text{var}[\hat{Y}_{t+k}(U_t, \ldots, U_{t+k-1})]\right\}.$$  

(6)
At the next time step $t + 1$, the previous expression is again evaluated for $U_{t+1}, \ldots, U_{t+k}$ with updated field state $X_{t+1}$ and newly observed constellation $Z_{t+1}$. The resultant optimal broadcast input $U^{*}_{t+1}$ is executed and the process is repeated. As the depth of future prediction $k$ is extended, the prediction error variance $\text{var}(Y_{t+k}) \leq E[(Y_{t+k} - E[Y_{t+k}])^2]$ increases. The prediction depth should be truncated before the prediction variance becomes too large to obtain a meaningful prediction. The variance can be evaluated empirically through simulation.

Optimization of (6) requires a significant amount of computation. However, the cell growth process is a very slow process, the sampling interval of which is of the order of 1 h. Comparing this to the latest ac servo control, i.e., 50 $\sim$ 100 $\mu$s, the cell growth process is more than $10^7$ times slower. This implies that using the same computer allows us to perform $10^7$ times more computations. Although the computation of (6) is heavy, multistep prediction can be executed in real time. The prediction depth $k$ must be selected by considering both computational complexity and the prediction error variance.

IV. SIMULATION

A. Simple System Dynamics

In this section, we simulate a simplified in vitro angiogenesis process and demonstrate that a cell population can be guided with broadcast control. We assume an ideal model for the matrix field dynamics. Specifically, the transport delay of growth factors over a matrix field is assumed to be much smaller than the time scale of cell growth. We also assume that the cells do not interfere with propagation of growth factors and the diffusion coefficient is uniform across the matrix field. Under these assumptions, the matrix field dynamics (2) reduces to an algebraic relation, and the state-transition probabilities become explicit functions of the input $U_t$.

VEGF is one of the most important and well-studied growth factors in angiogenesis. It is know that increased concentration of VEGF leads to greater probability of cell differentiation into a tip cell, and greater gradient leads to greater tip cell motility, facilitating vascular growth [9]. We use VEGF as a broadcast control medium, and supply it to an EC culture through two microfluidic channels, as shown in Fig. 1. Let $C_A$ and $C_B$ be concentrations of VEGF in microfluidic channels $A$ and $B$, respectively. Under the aforementioned assumptions on the matrix field, the VEGF concentration varies linearly across the matrix field between the two channels, and is given by

$$C(w) = C_B + mw, \quad \text{where} \quad m = \frac{(C_A - C_B)}{d}$$

(7)

where $C(w)$ is VEGF concentration at distance $w$ from chamber $B$ and $m$ is the gradient of VEGF concentration. Note that the two flow channel chamber allows us to control both VEGF concentration and gradient separately. Cells are seeded from chamber $B$, forming a monolayer at the bottom of the matrix field, as shown in Fig. 2. Sprouts develop from the monolayer and invade into the collagen gel matrix field, being led by tip cells. The tip cells dominate determination of sprout elongation, and branching occurs from any cell in the stalk of a sprout (but not from the tip). Probabilities of cell state transition $P_i = \{p_{ij}(X_i)\}$ are determined based on a simple model adapted from [4] and our in vitro experiments. Each tip cell decides to migrate with probability $p_M(m)$, and each stalk cell decides to branch out with probability $p_B(C)$, as given by linear saturation functions

$$p_M(m) = \begin{cases} \frac{m}{p_{max}^M}, & m < b \\ \frac{p_{max}^M}{p_{max}^M}, & m \geq b \end{cases}$$

and

$$p_B(C) = \begin{cases} \frac{C(w)}{a p_{max}^B}, & C(w) < a \\ \frac{p_{max}^B}{p_{max}^B}, & C(w) \geq a \end{cases}$$

(8)

where $p_{max}^M$ and $p_{max}^B$ are saturation probabilities for migration and branching, respectively, and $a$ and $b$ are scaling parameters. A migrating tip cell moves as a biased random walk in the direction of positive VEGF gradient with a probability $1 - 2p_R$ in the direction of the steepest gradient, and an equal probability $p_R$ for moving to either side of the gradient. Similar to [4], we assume that sprouts can be only form from the monolayer during the first time step. Stalk cells are assumed to proliferate and migrate as necessary to follow the tip, and occasionally, create a new branch with probability $p_B(C)$. For more details, see [2].

B. Feedback Control

We consider the following average branch length as a morphologic measure to regulate

$$Y_t = \frac{l_t}{N_t^B} \leq \frac{l_t}{N_t^B}$$

(9)

where $l_t$ is the total length of the vascular network and $N_t^B$ is the number of branches. When regulating $l_t$ to be at a desired value, $Y_t = y_d$, new branches tend to form as existing branches become too long. This forces the vascular network to spread out over the matrix field by extending sprouts in diverse directions, which is necessary for proper perfusion of blood to a tissue bed. While many measures could be used, average branch length is simple and serves to demonstrate the need for stochastic feedback control.

Traditionally, in vitro angiogenesis experiments are run open loop. Stimuli are fixed at certain levels and the process is allowed to evolve without intervention. Experimentally, we find that this leads to high variability in the network characteristics. Fig. 5(a) shows the results of an ensemble of 100 simulations run open loop with time courses $U_t$ selected to obtain, on average, $l_d = 10$, as shown. The figure shows that though $E[l_t]$ approaches $l_d$, the variance $\sigma^2 = \text{var}[l_t]$ increases with time, and the result after 100 time steps is highly variable. Fig. 5(b) shows the result of two-channel feedback control of VEGF concentration and gradient. The variance of $E[l_t]$ with feedback is significantly smaller than with open-loop control, yielding a much more consistent morphologic outcome. Note that the control law of (6) not only reduces the discrepancy of mean values, but also reduces the variance as expressed by the second term of the error norm. To regulate the average branch length and, thereby, cover the matrix field properly, the probabilities
of tip cell migration/extension and stalk cell branching need be coordinated. This requires that the VEGF concentration and gradient be proportioned properly. Fig. 6(a) shows the cell constellation when only the VEGF concentration was controlled actively when the gradient was kept at a high level. The tip cells migrated and extended too quickly with the high gradient, and branches were formed only near the tip cells after the sprouts had extended, leaving the area near the monolayer uncovered. In contrast, Fig. 6(b) shows the resultant constellation with a proper combination of VEGF concentration and gradient. A relatively small gradient allows the sprouts to branch out even near the monolayer, covering the area properly. This illustrates a coordinated control of VEGF concentration and gradient.

V. Conclusion

A systems-level description and control framework for guiding a population of ECs to a desired sprouting pattern formation in an in vitro microfluidic environment have been presented. Control of a cell population in the wet environment is broadcast in nature, and individual cell behaviors are stochastic. Nonetheless, stochastic optimal control is applicable and effective for producing a consistent branch pattern with low variance, as demonstrated with a simple simulation model. As more details of the sprouting angiogenesis model become available, the prediction accuracy and the control performance will be improved. The presented stochastic optimal control approach is general and applicable to a broad class of systems having diverse parameter values and model structure.

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