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HYBRID COMPUTATIONAL MODELING OF CELL POPULATION
AND MASS TRANSFER DYNAMICS IN TISSUE GROWTH
PROCESSES

by

GANG CHENG

A THESIS SUBMITTED
IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE

DOCTOR OF PHILOSOPHY

APPROVED, THESIS COMMITTEE

Kyriacos Zygourakis, Advisor
A. J. Hartsook Professor and Chair
Chemical and Biomolecular Engineering

Antonios G. Mikos
John W. Cox Professor, Bioengineering

Michael C. Gustin, Associate Professor
Biochemistry and Cell Biology

Nikolaos V. Mantzaris
Assistant Professor
Chemical and Biomolecular Engineering

Houston, Texas
April, 2005
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ABSTRACT

HYBRID COMPUTATIONAL MODELING OF CELL POPULATION AND MASS TRANSFER DYNAMICS IN TISSUE GROWTH PROCESSES

by

Gang Cheng

This work presents a comprehensive hybrid computer model simulating the cell population and mass transfer dynamics during tissue growth processes. The model has three major components: (a) a discrete algorithm simulating individual cell activities and cell-cell interactions; (b) transient, three-dimensional partial differential equations (PDE’s) describing the convection, diffusion, consumption and, possibly, secretion of nutrients or other important substances in tissue systems; and (c) equations describing how cell behavior is modulated by the local concentration fields.

The hybrid model is first used to study the growth of bioartificial tissues under conditions leading to nutrient depletion. Simulation results indicate that large tissue size, low nutrient diffusivity, high cell uptake rate and low nutrient concentration in the culture media lead to severe transport limitations and have serious adverse effects on the growth rates and the structure of bioartificial tissues. The incorporation of perfusion channels is one of the proposed methods for alleviating diffusional limitations. However, the selection of optimal channel placement and size leads to an interesting optimization problem. Our results indicate the existence of an
optimal channel diameter for each set of cell parameters and culture conditions. As diffusional limitations become more severe, larger perfusion channels are needed and the value of the achievable cell density decreases.

Finally, the hybrid model is used to study the acid-mediated growth of solid tumors. With its ability to describe the complex, three-dimensional vasculature of tissues invaded by tumors, our model represents a significant extension of previous two-dimensional studies. In addition to a three-dimensional capillary network generated from literature data, tree-like capillary networks with adjustable overall vascularity are generated using a bifurcating distributive algorithm in order to study the effect of host vascularity on tissue growth. Our simulations produce tumor growth curves similar to those observed clinically. The predicted range of tumor cell acid production rate shows better agreement with experimental values than existing two-dimensional models. Our model can also predict the universal existence of necrotic regions in large tumors.
ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to Professor Kyriacos Zygourakis for his invaluable guidance and support throughout the course of my research. I also thank him for giving me much freedom in exploring new areas of research interests.

I thank Professor Antonios G. Mikos, Professor Michael C. Gustin and Professor Nikolaos V. Mantzaris for serving in my thesis committee. Professor Pauline Markenscoff at the Electrical and Computer Engineering Department of University of Houston gave me many opinions on discrete modeling and parallel computing. I want to express my gratefulness to her.

Mr. Jian Feng in Professor Markenscoff’s group and I had discussions on parallel computing. Ms. Ailen Sanchez in Dr. Ka-Yiu San’s group helped me with the HPLC in their lab. Dr. Tanya Dunlap in the Cain Project proofread most of the chapters of this thesis. I do want to thank them.

I extend my appreciation to the Department of Chemical and Biomolecular Engineering here at Rice University for providing us with such an excellent academic environment. I also gratefully acknowledge the National Science Foundation for supporting my project financially.

Finally, I want to thank my parents who, though half a globe away, have always given me their encouragement and support. I would also like to thank my wife for always being here by my side during this long endeavor, no matter how endless it has appeared.
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CHAPTER 1  INTRODUCTION

1.1  Tissue Engineering

The loss or failure of an organ or tissue is one of the most frequent, devastating, and costly problems in health care. Conventional therapies include organ transplantation, reconstructive surgery, and implantation of mechanical devices (e.g., artificial kidneys). Although having saved and improved lives, these therapies remain imperfect solutions\cite{1, 2}. Transplantation is severely limited not only by a critical donor shortage, but also by the side effects, including weight gain, fluid retention, and elevated blood pressure, all of which are caused by the immunosuppressive drugs administered to prevent the host’s rejection of the transplanted organ or tissue\cite{3, 4}. Reconstructive surgery can result in undesirable biological changes, such as infection and hematoma, due to abnormal interaction of the tissue at its new location. Mechanical devices (like the artificial kidney) are also problematic because they cannot perform all the functions of a failing organ.

Over the last few decades, tissue engineering has emerged as a promising alternative to the aforementioned conventional therapies. It applies the knowledge gained in biology, biochemistry, medical sciences, and engineering to develop bioartificial implants or to induce tissue remodeling in order to replace, repair, or enhance the function of a particular tissue or organ\cite{1, 5}. The origins of tissue engineering can be traced back to
World War II when the discovery of many new synthetics encouraged the idea of improving on nature by using man-made materials\[6\]. Since then, the development in related fields of science and technology has considerably expanded the breadth of possibilities for fabricating living parts of the human body in the laboratory. The reconstitution of skin and arteries was first reported between 1979 and 1986 by E. Bell et al.\[6\]. Other living tissues, including cartilage, tendon, ligament, muscle, and bones, are focal points of current studies all over the world.

The basic procedure of tissue engineering is illustrated in Figure 1.1. After being harvested from a donor or another part of the patient’s body, the proper types of cells are seeded into a highly porous scaffold made either from natural proteins such as fibrin clots\[7\] and collagen gels\[8\], or from biodegradable synthetic polymers\[9\]. Besides serving as the structural component, the scaffolds provide the proper shape of the bioartificial tissue and a three-dimensional matrix for guided cell migration and proliferation. The cell-scaffold construct is then cultivated in a bioreactor with conditions (temperature, pH, humidity, mechanical stimulus, etc.) appropriately controlled until the tissue is suitable for implantation.

However, many difficulties must be overcome before we can engineer clinically useful bioartificial tissues using the previous procedure\[10\]. Tissue growth is a complex process whose rate and pattern are affected by many factors such as the cell phenotype, the density and spatial distribution of seed cells, and the culture conditions. Thus, the effective design of bioartificial tissues and the tissue culture method requires a comprehensive understanding of not only how cell-level properties are affected by external
conditions, but also how such changes may subsequently alter the rate and/or pattern of tissue growth through complicated population dynamics.

Figure 1.1: The basic procedure of tissue engineering.
1.2 Simulating Tissue Growth Processes

Recent advances in biomaterial research have made it possible for us to manipulate cellular functions (like adhesion and migration) through special fabrication techniques\textsuperscript{[11]} or biomimetic modification of biomaterial scaffolds\textsuperscript{[12-15]}. However, theoretical guidance on how altered cell-level properties may affect the process of tissue growth is still lacking. As the result, the development of tissue substitutes is still in an early stage, based almost exclusively on empirical approaches that require many expensive and time-consuming experiments.

We strongly believe that a systems-based approach and the development of computational models with predictive abilities will greatly speed up progress in this area. Such models will make it possible for us first to quickly assess the effect of various cell-level properties on tissue growth and then to manipulate controllable conditions in a rational way to alter such properties for the most desirable outcome. Computer models may one day become indispensable design tools for tissue engineers much the same way as they have been for automobile and airplane designers. Simulating tissue growth, however, is a very challenging task because of the overwhelming tissue-level biocomplexity which can be categorized mainly in three aspects.

First, considerable heterogeneity exists in cell populations. Cell division time, for example, distributes over a certain range instead of being the same for each cell in a population\textsuperscript{[16, 17]}. Many aspects of cell migration, such as speed and persistence time, also vary from cell to cell\textsuperscript{[18]}. It is thus necessary to differentiate each cell from others when simulating tissue
growth, a task not suitable for simple, deterministic models. Second, intricate population dynamics is involved in cell-cell interactions. Most anchorage-dependent mammalian cells stop dividing when completely surrounded by other cells, a phenomenon called contact inhibition\textsuperscript{[19, 20].} Such an effect of cell-cell interaction on cell division has been observed not only on flat surfaces\textsuperscript{[21-25]}, but also in three-dimensional scaffolds\textsuperscript{[26-29].} This makes cell migration an important factor for the growth of cell population because high cell motility helps to disperse cells and, thus, reduce the probability of cells being contact inhibited. Several studies found that the enhancement of cell proliferation rates resulting from the addition of growth factors to the culture media was actually caused by the growth factors’ stimulating effect on cell migration\textsuperscript{[30-32].} The migration of individual cells, however, can slowed down by cell-cell collisions which, depending on cell type, may cause a short pause in cell movement or the formation of an aggregate. Two fibroblasts, for example, will stop for about 25 minutes after a collision before breaking away from each other in new directions\textsuperscript{[33].} Similar phenomenon has been observed on bovine pulmonary artery endothelium cells\textsuperscript{[34, 35].} Quite differently, two epithelial cells adhere to each other after a collision and subsequent collisions with other cells result in the formation of small colonies and eventually a sheet of contiguous cells, a process essential for wound healing\textsuperscript{[33, 36]}.  

In addition to these two aspects of biocomplexity, tissues often grow under environmental conditions (e.g. local nutrient or growth factor concentrations, pH, etc.) can vary significantly with location inside the tissue. Such variation can be caused, as described next, by mass transfer
limitation of nutrients in bioreactors. Since the intracellular state strongly depends on local environmental conditions, cells exposed to spatially varying environmental conditions will exhibit spatially varying behavior.

1.3 Mass Transfer Limitation in Tissue Engineering

Tissue growth in vivo is supported by blood vessels which supply vital substances (e.g., nutrients and growth factors) for cell functions and also remove wastes (e.g., carbon dioxide and lactate) produced by cell metabolism. Most living tissue cells lie within about 1-3 cell widths of a capillary, the smallest blood vessels that exchange substances directly with cells. Capillary density varies considerably in the vascular beds of different organs, reflecting their metabolic demand: 2500–3000/mm³ in the brain, kidneys, liver, and myocardium; 300–400/mm³ in phasic units of the skeletal musculature; and < 100/mm³ in bone, fat, connective tissue, and in tonic units of the skeletal musculature[37]. The importance of blood vessels for tissue growth is also manifested in the process of angiogenesis – the proliferation of a network of blood vessels – which is vital for both wound healing[38, 39] and tumor metastasis[40, 41]. In fact, a new concept of cancer treatment based on the idea of blocking or diminishing angiogenesis is now being developed[42-44].

In the cell-scaffold construct cultivated in bioreactors, however, there are no blood vessels, and the only mode of mass transferring is passive diffusion. As a result, a nutrient concentration gradient exists in the scaffold
and becomes increasingly steeper as new cells and extracellular matrix (ECM) components are gradually produced at the scaffold-medium interface. Not only are nutrient diffusivities in the tissue (e.g., about $7.5 \times 10^{-11} \text{ m}^2/\text{s}$ at $37 \, ^{\circ}\text{C}$ for glucose$^{[45, 46]}$) significantly lower than in the medium (e.g., about $9.1 \times 10^{-10} \text{ m}^2/\text{s}$ at $37 \, ^{\circ}\text{C}$ for glucose$^{[47]}$), the nutrients are also constantly consumed by the cells. Thus, the thin layer of tissue forming first next to the scaffold surface creates a bottleneck effect that severely hinders the diffusion of nutrients into the scaffold interior. Studies have shown that such mass transfer limitation restricts tissue growth to regions less than a few hundred microns away from the scaffold surface$^{[48-50]}$. This problem is even more serious for tissues that normally have high vascularity and cellularity. For example, only very low thicknesses have been reported for engineered bones (250-500$\mu$m)$^{[51]}$ and cardiac tissues (50-180$\mu$m)$^{[48, 52, 53]}$. Such thin bioartificial tissues often cannot meet the requirement for a successful implantation.

### 1.4 Perfusion Tissue Culture System

Various methods have been tried to overcome, or at least to reduce, the mass transfer limitation during the cultivation of bioartificial tissues. Dynamic reactors such as mixed flasks and rotating vessels provide only partial solutions, producing tissue samples 2-3 mm in thickness$^{[48, 54-56]}$. One problem exists for rotating vessels in particular: the scaffolds collide frequently with the vessel walls during rotation, a movement that induces
cell damage and disrupts both cell attachment and the deposition of mineralized matrix in the scaffolds\cite{57, 58}. As a possible improvement, perfusion tissue culture systems were introduced in which pressure is applied to perfuse cell culture medium through the cell-scaffold construct (Figure 1.2A)\cite{49, 59-61}. By making the medium available for cells throughout the scaffold, such perfusion systems can produce tissues with much more uniform cell distribution. Moreover, the shear stress produced by the medium flow has been related in one study to the enhanced differentiation of and the increased mineralized matrix deposition by rat bone marrow stromal cells in three-dimensional scaffold\cite{62-64}. Unfortunately, however, it is precisely the shear stress that also limits the application of this tissue culture method to other types of cells. Although the response to shear stress depends on cell type\cite{65-67}, it has been reported that extended exposure to shear stress of 1.6-3.3 dyne/cm² in vitro altered the morphology and decreased the viability of various mammalian cells, including hybridoma and human embryonic kidney cells\cite{68, 69}. In their attempt to cultivate bioartificial cardiac tissue disks (2 mm in thickness, 5 mm in diameter) in a perfusion system like the one shown in Figure 1.2A, Carrier et al.\cite{49} found that even if the medium flow rate was kept so low (0.6-3.0 ml/min) that the estimated shear stress on cells was only 0.19-1.04 dyne/cm², most cells still exhibited a rounded phenotype, instead of the healthy flattened one. At such low flow rates, the perfused tissues could only reach an overall cell density comparable to that achievable in spinner flasks, even though the cell distribution was indeed more uniform. Given the relatively small tissue thickness, low medium flow rate, and already intermediate-level shear stress
in the Carrier et al. study, one can expect that cells will be subject to much higher shear stress if thicker (e.g., in centimeters) tissues are to be cultivated.

To reduce the impact of shear stress while still maintaining a relatively uniform distribution of cell culture medium in tissues, small parallel perfusion channels can be created in the tissue along the direction of medium flow to reduce the pressure drop (Figure 1.2B). Obviously, most of the medium will pass through the perfusion channels, as that is where the least resistance is. Hence, only cells at the tissue-channel interface will experience the shear stress created by the medium flow. The other cells will be shielded from this negative impact and the distance over which nutrients have to diffuse to reach them will be greatly reduced. In a sense, the perfusion channels are like “blood vessels” for the tissue. Although not yet widely adopted by tissue engineers, this channeled perfusion tissue culture method has shown great potential in a recent study by Kofidis et al.\cite{70}. In their study, a syngeneic rat aorta (mean diameter 1.8 mm) was embodied in the middle of a bioartificial tissue consisting of neonatal rat cardiomyocytes and fibrin glue. Continuous, pulsatile medium flow was then established through the aorta while the tissue was cultured in a circular chamber for 2 weeks. This approach produced highly viable, metabolically active, and mechanically stable cardiac tissues about 8 mm in thickness, a significant improvement over any previously reported results. Kofidis et al. also reported decreasing cell densities with increasing distance from the perfusion aorta, an indication that further improvement may be possible by embedding multiple uniformly distributed perfusion channels in the tissue.
The design of a multi-channel perfused bioartificial tissue then presents an optimization problem. When the channels are too small or too few, mass transfer limitations cannot be adequately overcome. When the channels are too big or too many, however, the volume of useful tissue is reduced too much and its mechanical integrity may also be at risk. For a given system (cell properties, nutrient diffusivity, etc.), there likely exists an optimal configuration of channel size and channel arrangement at which the highest overall cell density can be achieved after the tissue is cultivated for a given period of time. The determination of the optimal configuration, however, is not possible through merely qualitative estimations due to the aforementioned complexity of tissue growth processes and a systematic approach aided by computer models will be necessary.
Figure 1.2: The perfused bioartificial tissue in a bioreactor. (A) Conventional configuration. (B) Novel configuration with perfusion channels.
1.5 Research Objective

Our group has been working for several years on building discrete stochastic computer models that can simulate tissue growth processes. The model developed by Lee et al.\textsuperscript{[71]} was the first to directly quantify the competing effects of migration and contact inhibition without resorting to simplifying assumptions. This model followed the paths of individual cells as they executed persistent random walks on a two-dimensional grid, collided and proliferated to build a new tissue. Key parameters of this model could be easily obtained from long-term tracking and analysis of cell locomotion and division\textsuperscript{[34, 72]}. Simulation results agreed well with experimental data on the expansion of keratinocyte megacolonies\textsuperscript{[31]}, the growth of endothelium in culture media that contained varying concentrations of growth factors\textsuperscript{[71]} and the expansion of marrow stromal osteoblast megacolonies on biomimetic hydrogels\textsuperscript{[73]}. Cell migration speeds and the spatial distribution of seed cells were found to be crucial factors in determining proliferation rates. Because of the significant advantage they have in describing cell population dynamics, discrete models were also used in several recent studies to address the two-dimensional problems involving the aggregation and self-organization of \textit{Dictyostelium discoideum}\textsuperscript{[74-76]} and the interactions between extracellular matrix and fibroblasts\textsuperscript{[77]}. These studies were based on hybrid approaches that considered cells as discrete objects, while using continuous equations to describe the transport of regulatory molecules and signaling cascades.
The previous modeling approaches, however, have not been extended to three-dimensional tissue growth processes. Chang et al.\textsuperscript{[78]} developed a three-dimensional discrete model for the growth of biofilms, but the division of bacterial cells was not contact inhibited and cell migration was not considered. Kansal et al.\textsuperscript{[79]} developed a three-dimensional model based on cellular automata to simulate brain tumor growth dynamics. This model did not consider individual cells. Rather, each automaton represented 100 to $10^6$ real cells. While greatly reducing the computational load of simulations, this approach did not allow a detailed description of cell population dynamics.

This study will extend our earlier two-dimensional work by developing a hybrid model that describes the growth of three-dimensional tissues under conditions that may lead to significant mass transfer limitations. Our model will have the following three major components:

- A discrete stochastic model simulating individual cell activities and cell population dynamics.
- Three-dimensional partial differential equations (PDE’s) describing the diffusion-reaction processes in the tissue.
- Kinetic equations modulating individual cell activities according to local nutrient or growth factor concentrations.

The above major components of the model will be discussed separately in Chapters 2 – 4. They will then be combined in the study of the two practical problems. In Chapter 5, the model will be used to study the optimal design of perfused bioartificial tissues. To show the versatility of
our model, we will also use it to study the acid-mediated early-stage growth of solid tumors. The background and detailed analysis of that problem will be presented in Chapter 6. Finally, Chapter 7 will summarize the contributions of this study and discuss the possible directions for future work.
CHAPTER 2  SIMULATING CELL POPULATION DYNAMICS WITH CELLULAR AUTOMATA

2.1  Tissue Growth Dynamics

The growth of bioartificial tissues is a highly dynamic and complex process. In this section, we will focus our attention on cell population dynamics and develop a discrete model that describes the key cellular activities: migration, cell-cell collisions and proliferation. The schematic of Figure 2.1 illustrates these processes.

Migration of mammalian cells in uniform environments is most often described as a persistent random walk[80, 81]. Over short time periods, cells follow a relatively straight path, showing persistence of movement. If long time intervals are used to observe the cell position, however, cell movement appears similar to Brownian motion with frequent direction changes. Such migratory behavior has been observed on both flat surfaces and three-dimensional scaffolds[82-84]. If the position of the centroid of a cell is located at fixed time intervals, an "approximate" cell trajectory can be reconstructed which consists of many connected straight-line segments (Figure 2.1). A cell’s persistent random walk can be interrupted by breaks that are either spontaneous or caused by collisions with other cells. Depending on cell type, the duration of spontaneous breaks varies from a few minutes to many hours[72, 84, 85]. In the event of a cell-cell collision, both cells stop for a
certain length of time before resuming their movement in two random directions. While migrating and colliding, each cell goes through its division cycle at the end of which it stops to divide. The two new cells (called “daughter cells” hereafter) immediately move away from each other, also executing persistent random walks (dashed and dotted arrows in Figure 2.1). This process is repeated until confluence has been reached, that is until the scaffold is completely filled with cells.

While division is certainly important for the increase in cell number, migration and cell-cell collisions also play important roles by affecting the extent of contact inhibition which, as mentioned in Chapter 1, is exhibited by most anchorage-dependent mammalian cells. Higher migration speed, for example, shifts the onset of contact inhibition to higher values of cell density, thereby increasing tissue growth rate. A shorter stationary state after cell-cell collisions has a similar effect. A quantitative understanding of such relations, however, requires a systematic approach based on computer model simulations.
Figure 2.1: Schematic showing the tissue growth dynamics. Cells execute persistent random walks (solid, dashed and dotted arrows) while going through their division cycle. They may also collide.
2.2 Previous Work

Most of the early tissue growth models focused on the expansion of colonies of non-motile cells on flat surfaces. A deterministic model was proposed by Frame and Hu\cite{86}. The model tried to reflect the effect of contact inhibition by multiplying the exponential growth term by a coefficient term which decreases with increasing cell density. The main drawback of this model was that it could not reflect the difference made by cell’s specific position in the colony and, despite the fact that cells at the rim of the colony are free to divide, considered all cells to be equally contact inhibited. Another deterministic model was proposed by Cherry and Papoutsakis\cite{87}. It assumed that the cell growth rate was proportional to the number of cells in the perimeter of a colony, a more realistic description of the population dynamics. It did not, however, consider the merging of cell patches. Furthermore, the assumption of circular cell patches limited its applicability. Lim and Davies\cite{88} developed a stochastic model which treated each cell as irregular polygons with various number of sites growing over discrete time steps. Contact inhibition was implicitly realized by prohibiting cells from dividing if they were completely surrounded by other cells. A new class of discrete, stochastic model based on cellular automata (CA)\cite{89} was developed and experimentally validated by Zygourakis et al.\cite{90,91}. This model was shown to accurately describe the population dynamics during all stages of contact-inhibited cell proliferation. Similar approaches were later adopted by other researchers to simulate cell proliferation on microcarriers\cite{92,93}. 
All the above models are only applicable for non-motile cells. For motile cells, cell migration must be considered due to its important effect on cell proliferation. Cell migration was first introduced in a stochastic model proposed by Ruann et al.\textsuperscript{[94]}, but only for a duration of $1.5\bar{t}_d$ after a new cell is formed from a division ($\bar{t}_d$ is the average cell division time). A more general stochastic model that considered cell migration was reported by Lee et al.\textsuperscript{[71]}. This model described cell migration with parameters obtained from long-term tracking and analysis of cell locomotion\textsuperscript{[95]}. Simulation results agreed well with experimental data on the expansion of keratinocyte megacolonies\textsuperscript{[30, 31]}. In both of these models, average cell migration speed and spatial distribution of seed cells were found to be crucial for the expansion of cell colonies.

In spite of such exciting success of the two-dimensional stochastic CA models, this approach has not yet been fully extended to systems of practical importance that involve the growth of three-dimensional tissues consisting of migrating, dividing and interacting cells. Chang et al.\textsuperscript{[78]} developed a three-dimensional CA model for the growth of biofilms, but the division of bacterial cells was not contact inhibited, a property not shared by most anchorage-dependent mammalian cells. Moreover, cells in their model were non-motile. Another three-dimensional CA model was presented by Kansal et al.\textsuperscript{[79]} to simulate brain tumor growth dynamics. This model did not treat cells as individuals. Instead, each automaton represented 100 to $10^6$ real cells. While greatly reducing the computational load of simulations, this approach made it impossible to track the discrete history of each real cell. Cell migration was also not considered in their model.
2.3 Model Development

Since stochastic CA models have shown great potential in simulating cell population dynamics, we will use this approach to develop our tissue growth model. For this purpose, we need to first mathematically characterize the activities of individual cells and cell-cell interactions during tissue growth. We then describe how these processes can be simulated with cellular automata. Other details about the model including the initial conditions and its implementation on parallel computers will also be described.

The model developed in the following sections will be referred to as the CPMC/S/A model because it describes Cell Proliferation, Migration and Collision of a Single, Asynchronous population of cells that move with the same speed and persistence.

2.3.1 Characterization of Cell Activities

Migration

The persistent random walk of individual cells can be analyzed using a method based on the stochastic concept of Markov chains\textsuperscript{[34, 96, 97]}. Not only is this method compatible with the stochastic nature of cell migration, it is also suitable for analyzing both random and biased cell movement. To carry out the Markov chain analysis, we partition the three-dimensional space in which cells migrate into six subintervals: 1 = east, 2 = north, 3 = west, 4 = south, 5 = up and 6 = down (Figure 2.2). If a cell moves in a
direction that forms an angle $\theta$ with the $x$ axis and an angel $\varphi$ with the $xy$ surface, it will be in directional state 1 if $\theta \in (\pi/4, 3\pi/4]$ and $\varphi \in (-\pi/4, \pi/4]$, in directional state 2 if $\theta \in (3\pi/4, 5\pi/4]$ and $\varphi \in (-\pi/4, \pi/4]$, and so on. Additionally, a spontaneous stationary state (state 0) is defined according to the half cell diameter rule\textsuperscript{[97]}. That is, a cell is considered to be at the stationary state if it does not move more than half of a cell diameter between two successive time steps. With this definition of cell’s directional states, the following parameters can be extracted from cell trajectory data following the procedure described by Nobel and Levin\textsuperscript{[97]}:

- **Migration Speed**, $S$: this is the average speed of cell movement in the non-stationary states (i.e., states 1, 2, ..., and 6).
- **Persistence Time**, $E(T_k)$: the average time that a cell spends in directional state $k$ ($k = 0, 1, 2, ..., 6$). These parameters characterize the persistence of cell migration.
- **Transition Probabilities**, $p(l|k)$: the probability with which a cell turns from directional state $k$ to $l$ where $k = 0, 1, 2, ..., 6$, $l = 1, 2, ..., 6$ and $k \neq l$. These parameters characterize cells’ turning behavior. We should note that the sum of transition probabilities from one direction to all the other directions should be 1.0. That is, $\sum_{k=0}^{6} p(l|k) = 1.0$, where $l = 1, 2, ..., 6$ and $l \neq k$.

The magnitude of $S$ and $E(T_k)$ depends on both cell type and extracellular environment. Reported values range from $S = 0.5$ $\mu$m/min and $E(T_k) = 4-5$ hours for human microvessel endothelial cells and smooth
muscle cells$^{98, 99}$ to $S = 20$ μm/min and $E(T_k) = 4$ min for rabbit neutrophils$^{100}$. The transition probabilities are uniform in an isotropic environment, but factors such as substrate surface pattern and the presence of a chemoattractant can alter their values in one or several directions, reflecting biased cell movement.

Cell-Cell Collision

To account for the “slowdown” of cell migration caused by cell-cell collisions, we add another stationary state (state 7) to our model. Cells enter this state after a collision and will stay in the same location for a period of time equal to $E(T_7)$ before resuming their migration. The magnitude of $E(T_7)$ is a measure of the “stickiness” of cells, that is their tendency to form multi-cellular aggregates. Thus, we name $E(T_7)$ the “aggregation time”. Since a cell only enters directional state 7 after colliding with another one, the transition probabilities to this state are:

$$p(k|7) = \begin{cases} 1, & \text{if there is a collision} \\ 0, & \text{if there is no collision} \end{cases} \quad k = 0, 1, 2, \ldots, 7$$

When the aggregation time $E(T_7)$ has expired, the two cells move away from each other in randomly selected directions. Thus, the transition probabilities from direction 7 to other directions are:

$$p(7|l) = \begin{cases} 1, & \text{if } l = 1, 2, \ldots, 6 \\ \frac{1}{6}, & \text{if } l = 0 \text{ or } 7 \end{cases}$$
Division

Cell division is characterized in our model by the length $t_d$ of the division cycle in time. As mentioned in Chapter 1, $t_d$ usually distributes over a certain range in a population of cells. It is necessary, therefore, to specify cell division time with a discrete probability density function (PDF):

$$P_{\text{div}}([t_j, t_{j+1})) = \text{Probability that } t_j \leq t_d < t_{j+1}, j = 1, 2, \ldots, m$$

The PDF of $t_d$ can be measured using an assay described by Lee et al. in which the fraction of the population dividing between $t_j$ and $t_{j+1}$ ($j = 1, 2, \ldots, m$) are recorded using time-lapse video microscopy[Lee, 1994 #478].
Figure 2.2: The partition of a three-dimensional space into six equal subintervals for the analysis of persistent random walks using the Markov chains method.
2.3.2 The Cellular Automaton

A cellular automaton is an array of computational sites on a grid of specified shape that can exist in a finite number of possible states. The sites are usually called “cells”, but here we want to differentiate them from the actual cells. The state of each site with a finite number of possible states, on a grid of specified shape that evolves through discrete time steps according to a set of automaton rules governing the interaction between neighboring sites\cite{89, 101, 102}. To model the previously described dynamics of tissue growth, we use a cellular automaton consisting of a three-dimensional cellular grid with $N_x \times N_y \times N_z = N_t$ cubic computational sites. Each site can contain at most one cell and exists at one of a finite number of states at each time interval. That is, a site may be either

- **empty** and available for a cell to move in, or
- **occupied** by a cell that is at some point in its division cycle and either moves in a certain direction or is stationary.

Every site is “connected” to six immediate neighbors in the six directions defined in Figure 2.2 (von Neumann neighborhood\cite{103}) and its state evolves at discrete time steps $t^1, t^2, \ldots, t^r, t^{r+1}, \ldots$ (where $t^{r+1} = t^r + \Delta t$ for all $r$) as cells migrate, grow, divide and interact with each other. The control of cell migration speed is realized by allowing cells to move, if possible, from one site to an adjacent one over $n$ time steps while choosing the values of $n$ and $\Delta t$ so that
\[ S = \frac{h}{n \cdot \Delta t} \]

For a site \( i (i = 1, 2, \ldots, N) \) in the cellular grid, its state at \( t' \) \( x_i(t) \) is 0 if it is empty; otherwise, \( x_i(r) \) contains the following information that specifies the state of the cell currently occupying site \( i \):

- **Migration index, \( m_i \)**: If \( m_i = 1, 2, \ldots, 6 \), then the cell is migrating in one of the six directions (1=east, 2=north, 3=west, 4=south, 5=up and 6=down). If \( m_i = 0 \), the cell is stationary.

- **Division counter, \( k_{d,i} \)**: \( k_{d,i} = \frac{t_d}{\Delta t} \). This counter is decremented by one at each time step, and the cell divides when \( k_{d,i} = 0 \).

- **Persistence counter, \( k_{p,i} \)**: \( k_{p,i} = \frac{E(T_{m_i})}{\Delta t} \). This counter is decremented by one at each time step, and the cell turns when \( k_{p,i} = 0 \).

Let us assume that a cell \( i \) is in directional state \( k \) when its persistence counter \( k_{p,i} \) reaches zero. If there are empty sites in its immediate neighborhood, the cell will select one of them as its next location using the Monte Carlo method\(^{[104]}\) based on the probabilities \( p(k | l) \) of the possible transitions. If a cell is completely surrounded when \( k_{p,i} \) reaches zero, it cannot turn. Instead, it enters the stationary state \( k = 0 \). Its migration index is changed to zero and its persistence counter is reset to \( E(T_0) / \Delta t \). If the cell collides into another cell during its migration, both cells enter the stationary state \( k = 7 \) and their persistence counters are reset to \( E(T_7) / \Delta t \). When the
aggregation time expires, both cells can resume their migration in directions randomly selected using the Monte Carlo method.

Cell divisions are handled in a similar fashion. When the division counter $k_{d,i}$ reaches zero and there is at least one empty neighboring site, the cell will divide. One daughter cell will stay in the current site while the second one will be placed in a randomly selected neighboring site using again the Monte Carlo method. All free neighboring sites have equal probabilities of being selected. The two daughter cells are set to migrate in randomly selected directions and are assigned new division counters that are computed using the experimentally determined PDF of $t_d$. If a cell is completely surrounded when its division counter reaches zero, it cannot divide. Instead, it enters the stationary state $k = 0$ and its division counter is reset to start another division cycle.

### 2.3.3 Initial Conditions

Two initial conditions (seeding modes) are considered in our model to demonstrate the significant effect of the initial spatial distribution of cells on tissue growth. The first mode distributes $N_0$ seed cells randomly and uniformly throughout the cellular grid. This "Uniform" seeding mode is shown in Figure 2.3A, where the seed cells are depicted as small red cubes and the empty sites are made transparent to facilitate the visualization of the cell distribution. This is the most common seeding mode employed for in vitro culture of bioartificial tissues. Vunjak-Novakovic et al.\textsuperscript{[105]} used dynamic methods to seed bovine articular chondrocytes into polyglycolic
acid scaffolds and achieved volumetric seeding density in the range of 0.367~1.33% (calculated from data in the referred study while assuming that attached seed cells were, as shown by images in the same study, approximately 10 μm in diameter). Similar seeding densities have been reported for various combinations of cell type and scaffold material.\textsuperscript{[106-111]}

As we will see later, however, the uniform seeding mode may not be the most appropriate initial condition for studying the effect of surface modification on tissue growth. This is particularly true for scaffolds that are developed to promote wound healing, a serious problem with patients suffering from many debilitating diseases. To demonstrate the importance of initial conditions and, at the same time, show how our model can be used to optimize wound-healing therapies, we will consider a second seeding mode. In this “Wound” seeding mode, the seed cells occupy every site in the cellular grid surrounding a cylindrical “wound” (see Figure 2.3B). We will assume that this wound (the gray area in Figure 2.3B) is filled with a highly porous scaffold that allows cells to migrate freely in all directions. When the simulation starts, cells detach from the confluent tissue around the wound, migrate into the scaffold and proliferate to fill (or “heal”) the cylindrical wound. Simulations that start with this seeding mode will be referred to as “wound healing” runs hereafter.
Figure 2.3: The two initial conditions (seeding modes) used in our model: the “Uniform” seeding mode (Panel A) and the “Wound” seeding mode (Panel B). Sites occupied by seed cells are shown as small red cubes, while the empty sites are transparent in Panel A and gray in Panel B. The black line in Panel B indicates the initial shape of the “wound”.
2.3.4 Cell Population Dynamics

For every site $i$ $(1 \leq i \leq N_t)$ in the cellular automaton, the application of the rules described in the previous subsections defines a local transition function that specifies the state $x_i(r + 1)$ of the site at $t^{r+1} = t^r + \Delta t$ as a function of the state $x_i(r)$ at $t^r$ and the states of its six neighbors. The simultaneous application of the local transition functions to all the sites defines a global transition function $F$ that transforms a configuration $X(r) = [x_1(r), x_2(r), ..., x_{N_t}(r)]$ of the cellular automaton to the next one:

$$X(r + 1) = F[X(r)], \text{ where } r = 0, 1, 2, ...$$

Starting with the initial configuration $X(0)$, the global transition function $F$ transforms the cellular array to simulate the dynamic process of tissue growth. If at some time $t$ after the start of the simulation, $N_C(t)$ sites of the cellular automaton are occupied by cells, the cell volume fraction $\kappa(t)$ for runs starting with “Uniform” seeding is defined as

$$\kappa(t) = \frac{N_C(t)}{N_t}$$

For wound healing runs, we are interested in determining how fast the sites belonging to the cylindrical wound (equal to $N_t - N_0$) are filled with cells. For these runs, the cell volume fraction $\kappa_w(t)$ is defined by a slightly different formula:
\[ \kappa_w(t) = \frac{N_C(t) - N_0}{N_t - N_0} \]

As previously mentioned, spontaneous breaks in the persistent random walks and cell-cell collisions slow down the movement of cells. Thus, only a fraction of all the cells will move in the time interval \([t, t+\Delta t]\). We define the following effective speed of migration \(S_e\) for uniform seeding runs:

\[ S_e(t) = \frac{N_M(t)}{N_C(t)} \cdot S \]

where \(N_M(t)\) refers to the number of cells that were moving in the time interval \([t, t+\Delta t]\) and \(S\) is the cell migration speed described earlier. For wound healing runs, we first count the total number of cells \(N_{C,w}(t)\) and the number of migrating cells \(N_{M,w}(t)\) located inside the cylindrical wound. The effective speed of migration \(S_{e,w}\) is then calculated as

\[ S_{e,w}(t) = \frac{N_{M,w}(t)}{N_{C,w}(t)} \cdot S = \frac{N_{M,w}(t)}{N_C(t) - N_0} \cdot S \]

In both cases, the effective speed of a migration is a population-average quantity and is affected by: (a) the frequency and duration \(E(T_0)\) of spontaneous breaks in cell movement, (b) the frequency \(f_c(t)\) of cell-cell collisions and the magnitude of the aggregation time \(E(T_q)\), and (c) the fraction \(\varphi(t)\) of cells that are completely surrounded and, therefore, cannot
move. The frequency and duration of spontaneous breaks depend on the cell phenotype and the presence of soluble growth factors or ligands that modulate cell behavior. The frequency of collisions and the fraction of surrounded cells, however, depend primarily on the dynamics of the cell population. If we let $N_S(t)$ denote the number of completely surrounded cells in the time interval $[t, t + \Delta t]$, these two quantities can be computed as follows:

$$f_c(t) = \frac{\text{Number of collisions in interval } [t, t + \Delta t]}{N_C(t) \cdot \Delta t}$$

$$\phi(t) = \frac{N_S(t)}{N_C(t)}$$

For wound healing runs again, the frequency of collisions, $f_{c,w}(t)$, and the fraction of completely surrounded cells, $\phi_w(t)$, are found by considering only the “wound” sites for the computations of cell-cell collisions and surrounded cells.

### 2.3.5 Parallel Implementation of the Algorithm

The simulation of tissue growth is a computationally challenging problem requiring large grids to handle the populations of discrete cells and small time steps to accurately describe the cell population dynamics. For this reason, our tissue growth algorithm was parallelized for execution on distributed-memory multi-computers. The simulator uses the Message
Passing Interface (MPI)\textsuperscript{[113, 114]} for inter-processor communication (see detail of the algorithms in Appendix A). All runs discussed in this study were carried out on a Linux Networx Evolocity Cluster having 41 compute nodes and 1 front-end node. Each node has two 1.7Ghz Pentium IV Xeon processors and 2 GB of DDR memory. The nodes are connected using Myrinet, a switched 1.2GB/s network.

2.4 Parametric Studies

We show our model’s capability of simulating cell population dynamics by a series of parametric studies in which the effect of key model parameters on tissue growth is investigated. With the base case defined by the parameters listed in Table 1, only the value of one parameter will be changed in each study.

Images in Figure 2.4 are generated from the cell distribution data from a simulation run with the base case parameters. This process corresponds to the growth of a 1.2 mm×1.2 mm×1.2 mm tissue initially seeded with 1000 cells (0.1% seeding density) whose average diameter is 12 μm. Cells are allowed to migrate at 60 μm/hr and, if not contact inhibited, divide every 20 hr in average. The persistence time is 2.0 hr in directions 0, 1, ..., and 6. Colliding cells have to stay stationary for 1.0 hr before resuming their migrations. The transition probabilities are uniform and, thus, cells choose the next direction completely randomly when making a turn. As a result, the tissue grows in a uniform spatial pattern, with $\kappa(t)$ reaching 0.01, 0.1 and 0.5
after 3.23 days, 5.99 days and 7.90 days, respectively. We can learn more about cell population dynamics involved in the tissue growth process by examining the simulation results defined in Section 2.3.4. Figure 2.5A and Figure 2.5A describe the temporal evolution of $\kappa(t)$, $S_e$, $f_c(t)$ and $\varphi(t)$ for the process in Figure 2.4. We can see that after an initial lag phase, the tissue enters a stage of rapid growth reaching confluency after approximately 10 days. The effective speed of migration $S_e$ decreases with increasing cell density in the tissue (Figure 2.5A). The rate at which $S_e$ falls becomes more pronounced as the cell volume fraction goes above the 0.1 level. If we look at Figure 2.5B, we can see that this is primarily due to the increasing frequency of cell-cell collisions, events that force the colliding cells to enter a stationary state. $f_c(t)$ reaches a peak at about 8 days when $\kappa(t) \approx 0.50$. As the cell density increases beyond this level, we see a rapid increase in the fraction of completely surrounded cells that leads to a sharp drop in the frequency of actual cell-cell collisions.

The influence of cell population dynamics can be further revealed by the results of tissue growth rate $\frac{d\kappa(t)}{dt}$. In Figure 2.6, $\frac{d\kappa(t)}{dt}$ for the run in Figure 2.5 is compared to the results from two ideal cases. The curve labeled “Exponential” is generated from the following equation describing the exponential growth of suspension cells:

$$\frac{dN_c(t)}{dt} = \mu_{ana} \cdot N_c(t)$$

(2.1)

where $\mu_{ana}$ is the analytical specific growth rate whose value is
\[ \mu_{\text{ana}} = \frac{\ln(2)}{t_d} \]  

Eqn. (2.1) is valid only when every cell in the population is free to divide. That is, there is no contact inhibition. This simple model assumes an "average" division time for all cells and predicts that cells will start proliferating as soon as they are dispersed in the scaffold. Experimental studies have shown, however, that a significant time interval passes by before cells seeded on surfaces or in scaffolds will start proliferating\(^{[95]}\). To account for this initial delay, we have also developed a simple stochastic model that allows a population of cells to proliferate with the same distribution of division times used in our comprehensive model. The simple stochastic model does not account for migration or cell-cell collisions and assumes that all cells can divide freely up to the point where the population reaches confluence. Clearly, this model ignores contact inhibition effects and we will refer to it as the NCI (No-Contact-Inhibition) model.

Figure 2.6A and Figure 2.6B compare the cell fractions and growth rates predicted by our CPMC/S/A model to those predicted by the exponential growth model of Eq. (2.1) and the NCI discrete model. Contact inhibition effects become pronounced when the cell fraction rises above 0.2 \((t \approx 7 \text{ days})\). While both the exponential and the NCI models predict continuously increasing growth rates until the population reaches confluence, our model reveals that contact inhibition effects force \(d\kappa(t)/dt\) to pass through a maximum. To quantify the onset of significant contact
inhibition effects, we introduce the critical cell fraction \( \kappa^* \) as the cell fraction above which the predictions of the CPMC/S/A and NCI models differ by more than 5%. Specifically, if \( \kappa_s \) and \( \kappa_e \) are the cell fractions predicted by the CPMC/S/A and NCI models respectively, the critical cell fraction is defined by the following condition:

\[
\ln[\kappa_e(t)] - \ln[\kappa_s(t)] \geq 0.05 \ln[\kappa_s(t)] \quad \text{when } \kappa_s(t) \geq \kappa^*
\]

\( \kappa^* \) for the base case is about 0.167.

Table 1: The base-case parameters for the parametric studies on the CA model.

<table>
<thead>
<tr>
<th>Value</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>100×100×100</td>
<td>Size of cellular grid for the &quot;Uniform&quot; seeding mode</td>
</tr>
<tr>
<td>200×200×200</td>
<td>Size of cellular grid for the &quot;Wound&quot; seeding mode</td>
</tr>
<tr>
<td>100</td>
<td>Diameter of the wound for the &quot;Wound&quot; seeding mode</td>
</tr>
<tr>
<td>0.2</td>
<td>Time step of the simulation, hr</td>
</tr>
<tr>
<td>12</td>
<td>Average cell diameter, ( \mu \text{m} )</td>
</tr>
<tr>
<td>20</td>
<td>Average cell division time, hr</td>
</tr>
<tr>
<td>2</td>
<td>Variance in the normal distribution of cell division time, hr</td>
</tr>
<tr>
<td>60</td>
<td>Cell migration speed, ( \mu \text{m/hr} )</td>
</tr>
<tr>
<td>2.0</td>
<td>Persistence time in directional states 0, 1, 2, \ldots , and 6, hr</td>
</tr>
<tr>
<td>Uniform</td>
<td>Transition probabilities</td>
</tr>
<tr>
<td>1.0</td>
<td>Aggregation time after cell-cell collisions, hr</td>
</tr>
</tbody>
</table>
Figure 2.4: The base-case temporal evolution of a 100×100×100 cellular automaton simulating tissue growth in a uniformly seeded scaffold. The model parameters are listed in Table 1.
Figure 2.5: The base-case simulation results of the temporal evolution of cell volume fraction $\kappa(t)$, effective migration speed $S_e$, frequency of cell-cell collisions $f_c(t)$, and fraction of surrounded cells $\varphi(t)$. The model parameters are shown in Table 1.
Figure 2.6: The base-case simulation results of the temporal evolution of cell volume fraction $\kappa(t)$, and the tissue growth rate $d\kappa(t)/dt$. The model parameters are shown in Table 1.
Effect of Cell Division Time

As mentioned earlier, the CPMC/S/A model described here assigns to each cell a division time that is randomly selected from a normal distribution with mean $\bar{t}_d$ and variance $\sigma^2$. Figure 2.7 presents on a semilog plot of the temporal evolution of cell volume fractions for several runs where the mean division time $\bar{t}_d$ ranged from 8 hr to 24 hours with $\sigma^2$ fixed at 2 hours. For all these runs, a large cell migration speed ($S = 60 \ \mu$m/hr) is used to minimize the effect of contact inhibition. Figure 5 shows that the average cell division time is a key factor in determining tissue growth rates. The time required to reach confluence increases from 3.5 days for $\bar{t}_d = 8$ hours to 11 days for $\bar{t}_d = 24$ hours. Higher values of $\bar{t}_d$ prolong the initial delay in the growth curves (see Figure 5) because they shift the entire distribution of cell division times to the right. Changes in the variance $\sigma^2$ of the distribution of division time do not significantly affect the predicted results as long as the mean division time of the population is kept constant.

Effect of Migration Speed

The migration speed $S$ is a major indication of cell motility. Figure 2.8 and Figure 2.9 show the effect of $S$ on $\kappa(t)$ and $\varphi(t)$, respectively, in the “Uniform” seeding mode. It is clear that when cells move faster, the influence of contact inhibited can be delayed until there are more cells in the scaffold. This effect that can be characterized by the critical cell fraction $\kappa^*$ defined earlier. From the plot of $\kappa^*$ against $S$ in Figure 2.9, we can see that the benefit gained from increasing $S$ is very significant when $S$ is lower than 10 $\mu$m/hr (the time to reach confluence, $T_{\text{full}}$, is 23.33 days when $S = 0.0$
μm/hr and 10.37 days when \( S = 5.0 \text{ μm/hr} \), but it then quickly diminishes with increasing \( S \) (\( T_{\text{full}} \) is 9.82 days when \( S = 60.0 \text{ μm/hr} \)).

Cell migration speed has an even more significant effect on the healing of a wound. Figure 2.11 and Figure 2.12 show that for a wound whose size is specified in Table 1, the healing time, \( T_{\text{healing}} \), first decreases dramatically from 41.66 days when \( S = 0 \text{ μm/hr} \) to 8.47 days when \( S = 10 \text{ μm/hr} \), but then becomes much less sensitive to \( S \), only dropping to 4.47 days when \( S \) is as high as 60 μm/hr. In Figure 2.13, \( \varphi(t) \) is plotted against \( \kappa(t) \) for different values of \( S \). It is clear that when the number of cells in the wound area is the same, a higher fraction of cells are contact inhibited when \( S \) is smaller. This effect is directly illustrated by images in Figure 2.14 which describe the wound healing process for two of the cases in Figure 2.11: \( S = 1 \text{ μm/hr} \) (column 1) and \( S = 60 \text{ μm/hr} \) (column 2). We can see that because of the shape of the wound, only cells that have moved into the wound are not contact inhibited. Thus, higher migration speed helps the healing process by more efficiently “freeing” cells from the surrounding tissue in which they are completely surrounded.

Experimental studies have shown that in natural wound healing, migration speed of cells surrounding the wound is dramatically enhanced. Using time-lapse video microscopy, Chan et al.\(^ {115} \) monitored the migration and proliferation of rabbit corneal epithelial cells during \textit{in vitro} wound healing. The average migration speed of cells at wound edge was 104 μm/hr, significantly higher than its normal value of 30-40 μm/hr\(^ {116} \). A similar phenomenon has been observed by Zahm et al.\(^ {117} \) on the healing process of a wound created in a collagen I matrix cultured with human
respiratory epithelial cells. The simulations results presented above help to explain such natural phenomena by showing quantitatively how higher cell migration speed promotes wound healing.

**Effect of Transition Probabilities**

Transition probabilities characterize cells’ turning behavior. They are especially useful in simulating biased cell movement in situations such as chemotaxis\(^{[118-121]}\). Images in Figure 2.15 describe the growth of a tissue in an environment that is anisotropic for the directional preference of cell migration. In this example, \( p(l \mid j) = p_u^* \) for \( l = 1, 2, 3, 4, \) or \( 6, j = 0, 1, 2, \ldots, 5 \) and \( j \neq l \) while \( p(5 \mid j) = 5p_u^* \) for \( j = 0, 1, 2, 3, 4, \) or \( 6 \). That is, cells are 5 times more likely to turn upward than to any other directions. Comparing these images to those in Figure 2.4, we can see that in the isotropic environment, cells move randomly to all the possible directions and, thus, there is no special pattern in the way the tissue grows. When cells have a much higher probability of moving in one direction, however, the tissue develops gradually in the preferred direction, creating a propagating front of actively dividing cells while most of the other cells are contact inhibited. Results from a systematic study on the effect of transition probabilities are shown in Figure 2.16 in which the increasing bias in cell movement is expressed by the ration \( \lambda_s = \frac{p(5 \mid j)}{p_u^*} \). It is clear that as cell movement becomes more biased, tissue growth starts to slow down earlier and the curve deviates increasingly further away from the exponential growth curve. The evolution of \( \varphi(t) \) with \( \kappa(t) \) shown in Figure 2.17 reveals
that, like what we have seen when studying the effect of migration speed (Figure 2.9), the extent of contact inhibition is the fundamental reason for different tissue growth rate.

**Effect of Persistence Time**

Persistence time of cell migration has no significant effect on tissue growth rate in the "Uniform" seeding mode (data not shown), but plays an important role in wound healing. In Figure 2.18, $\kappa_w(t)$ is plotted against $t$ for a uniform persistence time $t_p = E(T_k)$ ($k = 0, 1, 2, \ldots, 6$) varying from 0.2 hr to 20 hr. The plot of $T_{healing}$ against $t_p$ in Figure 2.19 indicates that there exists an optimal $t_p$, $t_{p, opt}$, at which the wound heals most quickly. The distance that a cell migrates over $t_{p, opt}$ (about 8 hr in this example) is approximately $4/5$ of the radius of the cylindrical wound: distance covered = $8 \text{ hr} \times 60 \mu\text{m/hr} = 480 \mu\text{m}$ and wound radius = $50 \text{ cells} \times 12 \mu\text{m/cell} = 600 \mu\text{m}$. In Figure 2.20, the central section plane of the wound is visualized at several time points during the healing process for two of the cases in Figure 2.18: $t_p = 0.2 \text{ hr}$ (column 1) and $t_p = t_{p, opt} = 8 \text{ hr}$ (column 2). We can see that when $t_p$ is too short, cells stay close to the edge of the wound and, thus, the contact inhibition is only slightly reduced while when $t_p$ is too long, cells start off from one side of the wound, but do not stop until colliding into the opposite side, still not the most effective way of overcoming contact inhibition. Only when $t_p$ is equal to $t_{p, opt}$ can cells move away from one side of the wound without getting too close to the opposite side, a condition most desirable for bringing cells into the open space in the wound.
So far in experimental studies, the effect of persistence time has often coupled with the effect of migration speed. Lepekhin et al.[122] found that the different rates and patterns of wound healing in two-dimensional cultures of buccal, periodontal and skin fibroblasts were due to different migration speed and persistence time in these types of cells. Weimann et al.[123] studied the effect of Ca D-pantothenate on the healing of wounds created in cultures of human dermal fibroblasts and reported that Ca D-pantothenate accelerated the healing process by 1.2 ~ 1.6 fold by increasing cell migration speed and persistence time. Our model makes it possible for us to single out the effect of persistence time. The results presented above may provide guidance for the manipulation of persistence time for faster wound healing once it is possible to do so in reality.

**Effect of Aggregation Time**

As described before, the aggregation time $E(T_γ)$ is a measure of cells’ “stickiness”. Results in Figure 2.21 show that for both “Uniform” and “Wound” seeding modes, longer aggregation time slows tissue growth down. The effect is more significant in the “Wound” seeding mode because cell motility is more important for tissue growth in wound healing during which only cells in the wound area are free to divide.
Figure 2.7: The effect of average cell division time on tissue growth in the “Uniform” seeding mode. PDF of the cell division time is a normal distribution averaged at $t_d$ (shown in legend) with the variance $\sigma^2 = 2$ hr. The other model parameters are the same as in Table 1.
Figure 2.8: The effect of cell migration speed $S$ (shown in legend) on the temporal evolution of cell volume fraction $\kappa(t)$ in the “Uniform” seeding mode. The other model parameters are the same as in Table 1.
Figure 2.9: The Effect of cell migration speed $S$ (shown in legend) on the evolution of the fraction of surrounded cells $\varphi(t)$ with cell volume fraction $\kappa(t)$ in the "Uniform" seeding mode. The other model parameters are the same as in Table 1.
Figure 2.10: The effect of cell migration speed $S$ on the critical coverage $\kappa^*$ in the "Uniform" seeding mode. The other model parameters are the same as in Table 1.
Figure 2.11: The effect of cell migration speed $S$ (shown in legend) on the temporal evolution of cell volume fraction $\kappa_w(t)$ in the “Wound” seeding mode. The other model parameters are the same as in Table 1.
Figure 2.12: The effect of cell migration speed $S$ on the healing time $T_{healing}$ in the “Wound” seeding mode. The other model parameters are the same as in Table 1.
Figure 2.13: The effect of cell migration speed $S$ (shown in labels) on the evolution of the fraction of surrounded cells $\varphi(t)$ with cell volume fraction $\kappa_w(t)$ in the "Wound" seeding mode. The other model parameters are the same as in Table 1.
Figure 2.14: Images showing wound healing process for two of the cases in Figure 2.11: $S = 1 \mu m/hr$ (column 1) and $S = 60 \mu m/hr$ (column 2).
Figure 2.15: The growth of a tissue in an environment that is anisotropic for directional preference of cell migration. The model parameters are the same as in Table 1 except that the transition probability for cells to turn upward is 5 times higher than to any other directions.
Figure 2.16: The effect of biased cell migration (characterized by $\lambda_5$ which is defined in the text) on the temporal evolution of cell volume fraction $\kappa(t)$ in the “Uniform” seeding mode. The other model parameters are the same as in Table 1.
Figure 2.17: The effect of biased cell migration (characterized by $\lambda_5$ which is defined in the text) on the evolution of the fraction of surrounded cells $\varphi(t)$ with cell volume fraction $\kappa(t)$ in the "Uniform" seeding mode. The other model parameters are the same as in Table I.
Figure 2.18: The effect of persistence time $t_p$ (shown in legend) on the temporal evolution of cell volume fraction $\kappa_w(t)$ in the “Wound” seeding mode. The other model parameters are the same as in Table 1.
Figure 2.19: The effect of persistence time $t_p$ on the healing time $T_{healing}$ in the "Wound" seeding mode. The other model parameters are the same as in Table 1.
Figure 2.20: The central horizontal section planes showing wound healing process for two of the cases in Figure 2.18: $t_p = 0.2$ hr (column 1) and $t_p = 8$ hr (column 2).
Figure 2.21: The effect of aggregation time $E(T_T)$ (shown in legend) on the temporal evolution of cell volume fraction $\kappa(t)$ in the “Uniform” seeding mode. The other model parameters are the same as in Table 1.
Figure 2.22: The effect of aggregation time $E(T_\gamma)$ on the healing time $T_{\text{healing}}$ in the “Wound” seeding mode. The other model parameters are the same as in Table 1.
## 2.5 Summary

A discrete stochastic model based on cellular automata has been developed in this chapter to simulate cell population dynamics during tissue growth processes. The cellular automata approach captures the essential features of both individual cell activities and cell population dynamics. The included parameters (e.g., migration speed, persistence time, transition probabilities, and distribution of cell division time) allow a detailed description of cell migration and proliferation. Specific automaton rules are made to reflect the effect of intercellular interactions such as cell-cell collision and contact inhibition on the growth of the entire population. A random algorithm is used extensively to imitate the natural randomness in the above processes. The model allows various seeding modes, the most common of which being the “Uniform” seeding mode for *in vitro* tissue culture and the “Wound” seeding mode mimicking part of the wound healing process. The model has been implemented on parallel computers for higher efficiency and expanded capacity. Simulation results indicate that cell motility and the seeding mode have profound effects on cell population dynamics which further influence the rate and pattern of tissue development.

The heterogeneity in external stimuli such as nutrient concentrations is ignored in this chapter. It will need to be considered, however, if we are to develop a realistic tissue growth model for the two practical applications described in Chapter 1. Thus, we will proceed in the next chapter to solve the diffusion-reaction problem involved in tissue growth processes.
CHAPTER 3  SOLVING THE DIFFUSION-REACTION PROBLEM IN TISSUE SYSTEMS

3.1  General Diffusion-Reaction PDE

Transport limitations can lead to significant depletion of nutrients and soluble growth factors in the interior of three-dimensional scaffolds. These limitations can slow down cell proliferation and even lead to cell death in the scaffold interior\(^{124, 125}\). As mentioned in Chapter 1, this is the main reason that the maximum thickness of tissues grown in the laboratory rarely exceeds a fraction of a millimeter. Our ability to grow large tissues or organs will depend on whether we can increase nutrient availability in the scaffold interior either by flowing media through it or by pre-vascularizing the scaffold.

Simulations can be used to assess the effectiveness of such approaches. Increases in cell density accelerate local nutrient depletion, as the expanding cell population consumes increasingly larger amounts of nutrients. In the absence of forced convection (fluid flow through the scaffold), the nutrient concentration profile \(C(x,y,z,t)\) in the bioartificial tissue can be computed by solving a classical “diffusion-reaction” problem described by the following partial differential equation:

\[
\frac{\partial C}{\partial \tau} = \nabla \cdot [D_e \nabla (C)] - R(\rho_{\text{cell}}, C) + S(\rho_{\text{cell}}, C) + D(C) \tag{3.1}
\]
where $D_e$ is the effective diffusion coefficient, $\rho_{cell}$ is the local cell density, $R(\rho_{cell}, C)$ is the rate of cell-uptake, $S(\rho_{cell}, C)$ is the rate of secretion by the cells, $D(C)$ is the rate of natural degradation. We should note that for our discrete model, $\rho_{cell}$ is a discontinuous function reflecting the occupancy (by a cell) of the computational site ($x, y, z$) at time $t$ (see detail in Chapter 5). Eqn. (3.1) must be solved together with the appropriate initial condition:

$$C(x, y, z, 0) = C_0(x, y, z) \quad (3.2)$$

where $C_0(x, y, z)$ is a known concentration profile. In addition, the appropriate boundary conditions must be applied according to the geometry of the scaffold and the tissue culture conditions:

$$C = C_s \quad \text{on } \partial \Omega \quad \text{Dirichlet}$$
$$\frac{\partial C}{\partial n} = 0 \quad \text{on } \partial \Omega \quad \text{Neumann} \quad (3.3)$$

$$k_g (C_b - C_s) = D_e \frac{\partial C}{\partial n} \quad \text{on } \partial \Omega \quad \text{Mixed}$$

where $\partial \Omega$ refers to the outer surface of the scaffold, $\frac{\partial C}{\partial n}$ denotes the derivative with respect to the normal to the scaffold surface, $k_g$ is the external mass transfer coefficient, $C_b$ is the bulk concentration of the nutrient, and $C_s$ is the concentration of the nutrient at the outer surface of the scaffold. A convective term must be added to Eqn. (3.1) in the case of perfusion.
For small nutrient molecules (e.g., oxygen and glucose) that pass directly across the cell membrane, the kinetics of uptake and metabolism generally follow a Michaelis-Menten type dependence\cite{126}. The reaction term \( R(\rho_{\text{cell}}, C) \) in Eqn. (3.1) thus takes the following form:

\[
R(\rho_{\text{cell}}, C) = \rho_{\text{cell}} \frac{V_{\max} \cdot C}{K_m + C}
\]

(3.4)

where \( V_{\max} \) is the maximum cell-uptake rate and \( K_m \) is the saturation constant. Both \( V_{\max} \) and \( K_m \) can be measured through independent experiments. The secretion term \( S(\rho_{\text{cell}}, C) \) is zero since cells do not secret the nutrient. The natural degradation term \( D(C) \) is usually a first order function of \( C \) with the rate constant \( k_d \). Substituting these terms into Eqn. (3.1), we obtain the diffusion-reaction PDE for a nutrient:

\[
\frac{\partial C}{\partial t} = \nabla \cdot \left( D_e \cdot \nabla C \right) - \rho_{\text{cell}} \frac{V_{\max} \cdot C}{K_m + C} - k_d \cdot C
\]

(3.5)

The specific form of Eqn. (3.1) for lactic acid differs from Eqn. (3.5) in that its consumption term is zero while its secretion term for tumor cells is not (see detail in Chapter 6). The method of solving them, however, is the same and here we use Eqn. (3.5) as an example to describe it.

PDE’s are often nondimensionalized before being solved. The following dimensionless variables will be introduced for the nondimensionalization of Eqn. (3.5):
\[ \mu = \frac{C^*}{C^*}, \tau = \frac{t \cdot D_e^*}{(L^*)^2}, x = \frac{x}{L^*}, y = \frac{y}{L^*}, z = \frac{z}{L^*}, \delta = \frac{D_e}{D_e^*}, \rho_{cell} = \frac{\rho_{cell}^*}{\rho_{cell}^*} \]

\[ \phi^2 = \frac{D_e^* C^* V_{\max} (L^*)^2}{D_e^* C^*}, \beta = \frac{K_m}{C^*}, \xi = \frac{k_d (L^*)^2}{D_e^*} \]

where \( C^* \), \( D_e^* \), \( L^* \) and \( \rho_{cell}^* \) are the characteristic concentration, effective diffusivity, length, and cell density, respectively. The dimensionless number \( \phi^2 \) above is called the Thiele modulus which is an indication of the relative magnitude of the reaction term over the diffusion term\(^{[127]}\). It will be referred to in Chapter 5 to identify the factors that lead to severe mass transfer limitations. Using the above dimensionless numbers, Eqn. (3.5) can be re-written as

\[ \frac{\partial \mu}{\partial \tau} = \nabla (\delta \cdot \nabla \mu) - \rho_{cell} \frac{\phi^2 \mu}{\beta + \mu} - \xi \cdot \mu \]  \( (3.6) \)

### 3.2 Discretization

PDE’s such as Eqn. (3.6) do not have analytical solutions and have to be solved numerically. In order to do that, we first need to discretize them over a certain grid which in our case is the cellular grid established in the CA model in Chapter 2. Discretization of PDE’s should be done with care. A good scheme is often a compromise between efficiency and stability. The diffusive terms are typically stiff and should thus be integrated implicitly to
avoid excessively small time steps. An implicit treatment of the non-linear reaction terms could, however, be undesirable because the Jacobian of this term is typically nonsymmetrical or indefinite. Therefore, Implicit-Explicit (IMEX) schemes are often used to discretize diffusion-reaction PDE’s by applying an implicit treatment to the diffusive term and an explicit treatment to the reaction term(s)\(^{128}\). The resulting system can be solved efficiently with iterative techniques. Some of the most widely used IMEX schemes include the second-order semi-implicit backward differentiation formula (2-SBDF) method, Crank-Nicolson Adams-Bashforth (CNAB) method, and modified CNAB (MCNAB) method. Among them, MCNAB has been shown to have a small truncation error and to give a good approximation of high frequency spatial modes\(^{129}\). We now describe the discretization of Eqn. (3.6) with the MCNAB method.

We assume that \(d\bar{x}, d\bar{y},\) and \(d\bar{z}\) are the grid sizes in \(x, y\) and \(z\) directions, respectively, and \(d\tau\) is the time step. For convenience, the following abbreviation is introduced to denote the concentration at point \((i, j, k)\) at time \(\tau = m \cdot d\tau\):

\[
\mu_{i,j,k}^m = \mu((i-1)d\bar{x}, (j-1)d\bar{y}, (k-1)d\bar{z}, md\tau)
\]

With the 7-point finite difference method\(^{130}\) that we use, \(\mu_{i,j,k}^m\) is approximated as a function of \(\mu_{i,j,k}^m\) and the concentrations of the 6 neighboring grid points (Figure 1.1) at \(m \cdot d\tau\) and \((m+1) \cdot d\tau\).
Figure 3.1: The relative position of neighboring grid points (filled circles) in the concentration grid. $\delta_1 - \delta_6$ are the nondimensionalized effective diffusivities in the middle of two points in the six directions, respectively.
\[
\frac{\mu_{i,j,k}^{m+1} - \mu_{i,j,k}^m}{d\tau} \cdot dx dy dz = \left[ \frac{\delta_3(\mu_{i-1,j,k}^{m+1} - \mu_{i,j,k}^{m+1}) - \delta_1(\mu_{i,j,k}^{m+1} - \mu_{i+1,j,k}^{m+1})}{dx} \right] \cdot dy dz \\
+ \left[ \frac{\delta_4(\mu_{i,j-1,k}^{m+1} - \mu_{i,j,k}^{m+1}) - \delta_2(\mu_{i,j,k}^{m+1} - \mu_{i,j+1,k}^{m+1})}{dy} \right] \cdot dx dz \\
+ \left[ \frac{\delta_5(\mu_{i,j,k-1}^{m+1} - \mu_{i,j,k}^{m+1}) - \delta_5(\mu_{i,j,k}^{m+1} - \mu_{i,j,k+1}^{m+1})}{dz} \right] \cdot dx dy \\
- R(\rho_{cell}, \mu_{i,j,k}^{m+1}) \cdot dx dy dz - D(\mu_{i,j,k}^{m+1}) \cdot dx dy dz
\]

(3.7)

where \( R(\rho_{cell}, \mu_{i,j,k}^{m+1}) = \rho_{cell} \frac{\phi^2 \mu_{i,j,k}^{m+1}}{\beta + \mu_{i,j,k}^{m+1}} \) and \( D(\mu_{i,j,k}^{m+1}) = \xi \cdot \mu_{i,j,k}^{m+1} \). If we let

\[
\Phi_{i,j,k}^m = \frac{\delta_3(\mu_{i-1,j,k}^m - \mu_{i,j,k}^m) - \delta_1(\mu_{i,j,k}^m - \mu_{i+1,j,k}^m)}{(dx)^2} \\
+ \frac{\delta_4(\mu_{i,j-1,k}^m - \mu_{i,j,k}^m) - \delta_2(\mu_{i,j,k}^m - \mu_{i,j+1,k}^m)}{(dy)^2} \\
+ \frac{\delta_5(\mu_{i,j,k-1}^m - \mu_{i,j,k}^m) - \delta_5(\mu_{i,j,k}^m - \mu_{i,j,k+1}^m)}{(dz)^2}
\]

and then apply the MCNAB method to Eqn. (3.7), we will have
\[
\frac{\mu_{i,j,k}^{m+1} - \mu_{i,j,k}^m}{d\tau} = \left( \frac{9}{16} \Phi_{i,j,k}^{m+1} + \frac{3}{8} \Phi_{i,j,k}^m + \frac{1}{16} \Phi_{i,j,k}^{m-1} \right) \\
- \left[ \frac{3}{2} R\left( \rho_{cell}, \mu_{i,j,k}^m \right) - \frac{1}{2} R\left( \rho_{cell}, \mu_{i,j,k}^{m-1} \right) \right] \\
- \xi \left( \frac{9}{16} \mu_{i,j,k}^{m+1} + \frac{3}{8} \mu_{i,j,k}^m + \frac{1}{16} \mu_{i,j,k}^{m-1} \right)
\]

Replacing the abbreviation of the second-order terms, we will get

\[
\frac{\mu_{i,j,k}^{m+1} - \mu_{i,j,k}^m}{d\tau} = \frac{9}{16} \left\{ \frac{\delta_3}{(d x)^2} \mu_{i-1,j,k}^{m+1} + \frac{\delta_1}{(d x)^2} \mu_{i+1,j,k}^{m+1} + \frac{\delta_4}{(d y)^2} \mu_{i,j-1,k}^{m+1} \\
+ \frac{\delta_2}{(d y)^2} \mu_{i,j+1,k}^{m+1} + \frac{\delta_6}{(d z)^2} \mu_{i,j,k-1}^{m+1} + \frac{\delta_5}{(d z)^2} \mu_{i,j,k+1}^{m+1} \right\} \\
+ \left[ \frac{\delta_3 + \delta_1}{(d x)^2} + \frac{\delta_4 + \delta_2}{(d y)^2} + \frac{\delta_6 + \delta_5}{(d z)^2} \right] \mu_{i,j,k}^{m+1} \\
+ \frac{3}{8} \Phi_{i,j,k}^m + \frac{1}{16} \Phi_{i,j,k}^{m-1} \right\} \right) \\
- \xi \left( \frac{9}{16} \mu_{i,j,k}^{m+1} + \frac{3}{8} \mu_{i,j,k}^m + \frac{1}{16} \mu_{i,j,k}^{m-1} \right)
\]

Moving all the unknown terms at \((m+1)\cdot d\tau\) to the left-hand side of the equation and the other terms to the right-hand side, we will obtain
\[
\left\{1 + \frac{9}{16} d\tau \left[ \frac{\delta_3 + \delta_1}{(d\tau)^2} + \frac{\delta_4 + \delta_2}{(d\tau)^2} + \frac{\delta_6 + \delta_5}{(d\tau)^2} + \xi \right] \right\} \mu_{i,j,k}^{m+1}
\]
\[
- \frac{9}{16} d\tau \left[ \frac{\delta_3}{(d\tau)^2} \mu_{i-1,j,k}^{m+1} + \frac{\delta_1}{(d\tau)^2} \mu_{i+1,j,k}^{m+1} + \frac{\delta_4}{(d\tau)^2} \mu_{i,j-1,k}^{m+1} 
+ \frac{\delta_2}{(d\tau)^2} \mu_{i,j+1,k}^{m+1} + \frac{\delta_6}{(d\tau)^2} \mu_{i,j,k-1}^{m+1} + \frac{\delta_5}{(d\tau)^2} \mu_{i,j,k+1}^{m+1} \right] \right] 
\]
\[
= \mu_{i,j,k}^m + d\tau \left[ \frac{3}{8} \Phi_{i,j,k}^m + \frac{1}{16} \Phi_{i,j,k}^{m-1} - \frac{3}{2} R(\rho_{cell}^m, \mu_{i,j,k}^m) + \frac{1}{2} R(\rho_{cell}^m, \mu_{i,j,k}^{m-1}) \right]
\]
\[- \frac{3}{8} \xi \cdot \mu_{i,j,k}^m - \frac{1}{16} \xi \cdot \mu_{i,j,k}^{m-1} \right] \right]
\]

If we then let
\[
e_1 = \frac{9}{16} d\tau \frac{\delta_1}{(d\tau)^2}, e_2 = \frac{9}{16} d\tau \frac{\delta_2}{(d\tau)^2}, e_3 = \frac{9}{16} d\tau \frac{\delta_3}{(d\tau)^2}
\]
\[
e_4 = \frac{9}{16} d\tau \frac{\delta_4}{(d\tau)^2}, e_5 = \frac{9}{16} d\tau \frac{\delta_5}{(d\tau)^2}, e_6 = \frac{9}{16} d\tau \frac{\delta_6}{(d\tau)^2}
\]
\[
a = 1 + \sum_{i=1}^{6} e_i + \frac{9}{16} \xi \cdot d\tau \right]
\]
\[
b_{i,j,k}^{m+1} = \mu_{i,j,k}^m + d\tau \left[ \frac{3}{8} \Phi_{i,j,k}^m + \frac{1}{16} \Phi_{i,j,k}^{m-1} - \frac{3}{2} R(\rho_{cell}^m, \mu_{i,j,k}^m) + \frac{1}{2} R(\rho_{cell}^m, \mu_{i,j,k}^{m-1}) \right]
\]
\[- \frac{3}{8} \xi \cdot \mu_{i,j,k}^m - \frac{1}{16} \xi \cdot \mu_{i,j,k}^{m-1} \right] \right]
\]

we will get
\[
a \cdot \mu_{i,j,k}^{m+1} = \left( e_3 \cdot \mu_{i-1,j,k}^{m+1} + e_1 \cdot \mu_{i+1,j,k}^{m+1} + e_4 \cdot \mu_{i,j-1,k}^{m+1} + e_2 \cdot \mu_{i,j+1,k}^{m+1} 
+ e_6 \cdot \mu_{i,j,k-1}^{m+1} + e_5 \cdot \mu_{i,j,k+1}^{m+1} \right) = b_{i,j,k}^{m+1} \tag{3.8}
\]
For a certain time step, all the unknown concentrations of the next time step are now on the left-hand side of Eqn. (3.8), and the known concentrations are on the right-hand side. For an $N_x \times N_y \times N_z$ cellular grid, we can write Eqn. (3.8) following the sweeping order first in $x$ direction, then in $y$ and finally in $z$ directions. The resulting set of equations can be transformed into the matrix-vector form:

$$A^{N \times N} \cdot x^N = b^N$$  \hspace{1cm} (3.9)

where $A^{N \times N}$ ($N = N_x \times N_y \times N_z$) is a sparse coefficient matrix with non-zero diagonal elements and no more than 6 non-zero off-diagonal elements in each row, $x^N$ is the vector of unknown concentrations, and $b^N$ is the vector calculated from the concentrations at the two previous time steps.

### 3.3 Boundary Conditions

#### 3.3.1 Dirichlet Boundary Condition

When a tissue is cultured in a well-mixed bioreactor, it is possible that mass transfer between tissue surfaces and the bulk of the medium is so efficient that concentrations at the two sides are approximately the same. If so, the Dirichlet boundary condition shown below can be applied to Eqn. (3.5).
\[ C = C_b \]  

(3.10)

where \( C_b \) is the bulk concentration of the nutrient which is assumed to be constant. Eqn. (3.10) can be nondimensionalized as

\[ \mu = \mu_b, \text{ where } \mu_b = \frac{C_b}{C^*} \]  

(3.11)

For grid points on the boundary, therefore, Eqn. (3.8) becomes \( \mu_{i,j,k}^{m+1} = \mu_b \). The only non-zero element in this row of the coefficient matrix \( A \) in Eqn. (3.9) is then the diagonal element which is equal to 1, and the element in the same row of the right-hand-side vector \( b \) should be \( \mu_b \).

3.3.2 Mixed Boundary Condition

When the mass transfer from the bulk of the medium to tissue surfaces is not efficient enough for Dirichlet boundary condition to be applicable, the mixed boundary condition is often used:

\[ D_e \frac{\partial C}{\partial n} = k_g (C_b - C_s) \text{ on the surface} \]  

(3.12)

where \( k_g \) is the mass transfer coefficient from the medium to the tissue, \( C_b \) is the bulk concentration of the nutrient, and \( C_s \) is the nutrient concentration on the tissue side of the surface. Eqn. (3.12) can be nondimensionalized as
\[ \delta \cdot \frac{\partial \mu}{\partial n} = Bi \cdot (\mu_b - \mu) \text{ on the surface} \quad (3.13) \]

where \( Bi = \frac{k_s L^*}{D_e^*} \) is usually called the Biot number which relates the mass transfer resistance inside and at the surface of a body\cite{127}.

For PDE discretization, the mixed boundary condition can be utilized by assuming the existence of an imaginary grid point on the medium side of the tissue-medium interface. If, for example, the surface where \( i = 1 \) has the mixed boundary condition, we assume that there exists an imaginary grid point \((0, j, k)\) as shown in Figure 3.2A. The Taylor extension of \( \mu_{0,j,k} \) is then

\[ \mu_{0,j,k} = \mu_{1,j,k} - \frac{\partial \mu}{\partial x_{1,j,k}} \cdot d^2 x + \frac{\partial^2 \mu}{\partial x^2_{1,j,k}} \frac{(d^2 x)^2}{2!} - \frac{\partial^3 \mu}{\partial x^3_{1,j,k}} \frac{(d^3 x)^3}{3!} + \ldots \quad (3.14) \]

and the same extension of \( \mu_{2,j,k} \) is

\[ \mu_{2,j,k} = \mu_{1,j,k} + \frac{\partial \mu}{\partial x_{1,j,k}} \cdot d^2 x + \frac{\partial^2 \mu}{\partial x^2_{1,j,k}} \frac{(d^2 x)^2}{2!} + \frac{\partial^3 \mu}{\partial x^3_{1,j,k}} \frac{(d^3 x)^3}{3!} + \ldots \quad (3.15) \]

Eqn. (3.15)- Eqn. (3.14) gives

\[ \frac{\partial \mu}{\partial x_{1,j,k}} = \frac{\mu_{2,j,k} - \mu_{0,j,k}}{2d x} + O(d^3 x) \]
Ignoring the third-order term, we get

\[ \mu_{0,j,k} \approx \mu_{2,j,k} - 2d_x \cdot \frac{\partial \mu}{\partial x_{1,j,k}} \]

From Eqn. (3.13), we have \( \frac{\partial \mu}{\partial x_{1,j,k}} = -\nabla_n \mu = -\frac{Bi}{\delta_1} (\mu_{\text{bulk}} - \mu_{1,j,k}) \), which can be rearranged into

\[ \mu_{0,j,k} = \mu_{2,j,k} + \frac{2d_x \cdot Bi}{\delta_1} (\mu_{\text{bulk}} - \mu_{1,j,k}) \]  

(3.16)

For \( i = 1 \), Eqn. (3.8) becomes

\[ a \cdot \mu_{1,j,k}^{m+1} = \left( e_3 \cdot \mu_{0,j,k}^{m+1} + e_1 \cdot \mu_{2,j,k}^{m+1} + e_4 \cdot \mu_{1,j-1,k}^{m+1} + e_2 \cdot \mu_{1,j+1,k}^{m+1} + e_6 \cdot \mu_{1,j,k-1}^{m+1} + e_5 \cdot \mu_{1,j,k+1}^{m+1} \right) = b_{1,j,k}^{m+1} \]

Applying Eqn. (3.16) gives

\[ a \cdot \mu_{1,j,k}^{m+1} = \left( e_1 \cdot \mu_{2,j,k}^{m+1} + \frac{2d_x \cdot Bi \cdot e_1}{\delta_1} (\mu_{\text{bulk}} - \mu_{1,j,k}^{m+1}) + e_4 \cdot \mu_{1,j-1,k}^{m+1} + e_2 \cdot \mu_{1,j+1,k}^{m+1} + e_6 \cdot \mu_{1,j,k-1}^{m+1} + e_5 \cdot \mu_{1,j,k+1}^{m+1} \right) = b_{1,j,k}^{m+1} \]

which can be rearranged into
\[
\left( a + \frac{2d^- x \cdot \sigma \cdot e_1}{\delta_1} \right) \cdot \mu^{m+1}_{1,j,k} - \left( 2e_1 \cdot \mu^{m+1}_{2,j,k} + e_4 \cdot \mu^{m+1}_{1,j-1,k} + e_2 \cdot \mu^{m+1}_{1,j+1,k} \\
+ e_6 \cdot \mu^{m+1}_{1,j,k-1} + e_5 \cdot \mu^{m+1}_{1,j,k+1} \right) \right)
= b^{m+1}_{1,j,k} + \frac{2d^- x \cdot Bi \cdot e_1}{\delta_1} \mu_{bulk} 
\]

(3.17)

If the surface where \( i = N_x \) has the mixed boundary condition, we assume that there exists an imaginary grid point \((N_x+1, j, k)\) as shown in Figure 3.2B. The Taylor extension of \( \mu_{N_x, j, k} \) is then

\[
\mu_{N_x, j, k} = \mu_{N_x, j, k} - \frac{\partial \mu}{\partial x_{N_x, j, k}} \cdot d^- x + \frac{\partial^2 \mu}{\partial x^2_{N_x, j, k}} \frac{(d^- x)^2}{2!} + \frac{\partial^3 \mu}{\partial x^3_{N_x, j, k}} \frac{(d^- x)^3}{3!} + ... \]  
(3.18)

and the same extension of \( \mu_{N_x+1, j, k} \) is

\[
\mu_{N_x+1, j, k} = \mu_{N_x, j, k} + \frac{\partial \mu}{\partial x_{N_x, j, k}} \cdot d^- x + \frac{\partial^2 \mu}{\partial x^2_{N_x, j, k}} \frac{(d^- x)^2}{2!} + \frac{\partial^3 \mu}{\partial x^3_{N_x, j, k}} \frac{(d^- x)^3}{3!} + ... \]  
(3.19)

Eqn. (3.19) – Eqn. (3.18) gives

\[
\frac{\partial \mu}{\partial x_{N_x, j, k}} = \frac{\mu_{N_x+1, j, k} - \mu_{N_x-1, j, k}}{2d^- x} + O(d^- x)^3
\]

Ignoring the third-order term, we get

\[
\mu_{N_x+1, j, k} \approx \mu_{N_x-1, j, k} + 2d^- x \cdot \frac{\partial \mu}{\partial x_{N_x, j, k}}
\]
From Eqn. (3.13), we have \( \frac{\partial \mu}{\partial x_{N_x,j,k}} = \nabla_n \mu = \frac{Bi}{\delta_3} \left( \mu_{bulk} - \mu_{N_x,j,k} \right) \) which can be rearranged into

\[
\mu_{N_x+1,j,k} = \mu_{N_x-1,j,k} + \frac{2d \bar{x} \cdot Bi}{\delta_3} \left( \mu_{bulk} - \mu_{N_x,j,k} \right)
\]  

(3.20)

For \( i = N_x \), Eqn. (3.8) becomes

\[
a \cdot \mu_{N_x,j,k}^{m+1} - \left( e_3 \cdot \mu_{N_x-1,j,k}^{m+1} + e_1 \cdot \mu_{N_x+1,j,k}^{m+1} + e_4 \cdot \mu_{N_x,j-1,k}^{m+1} + e_2 \cdot \mu_{N_x,j+1,k}^{m+1} + e_6 \cdot \mu_{N_x,j,k-1}^{m+1} + e_5 \cdot \mu_{N_x,j,k+1}^{m+1} \right) = b_{N_x,j,k}^{m+1}
\]

Applying Eqn. (3.20) gives

\[
a \cdot \mu_{N_x,j,k}^{m+1} - \left( e_3 \cdot \mu_{N_x-1,j,k}^{m+1} + e_3 \cdot \mu_{N_x+1,j,k}^{m+1} + \frac{2d \bar{x} \cdot Bi \cdot e_3}{\delta_3} \left( \mu_{bulk} - \mu_{N_x,j,k}^{m+1} \right) + e_4 \cdot \mu_{N_x,j-1,k}^{m+1} + e_2 \cdot \mu_{N_x,j+1,k}^{m+1} + e_6 \cdot \mu_{N_x,j,k-1}^{m+1} + e_5 \cdot \mu_{N_x,j,k+1}^{m+1} \right) = b_{N_x,j,k}^{m+1}
\]

which can be rearranged into

\[
\left( a + \frac{2d \bar{x} \cdot \sigma \cdot e_3}{\delta_3} \right) \mu_{N_x,j,k}^{m+1} - \left( 2e_3 \cdot \mu_{N_x-1,j,k}^{m+1} + e_4 \cdot \mu_{N_x,j-1,k}^{m+1} + e_2 \cdot \mu_{N_x,j+1,k}^{m+1} + e_6 \cdot \mu_{N_x,j,k-1}^{m+1} + e_5 \cdot \mu_{N_x,j,k+1}^{m+1} \right) = b_{N_x,j,k}^{m+1} + \frac{2d \bar{x} \cdot Bi \cdot e_3}{\delta_3} \mu_{bulk}
\]  

(3.21)
Figure 3.2: Imaginary concentration grid points are created in order to utilize the mixed boundary conditions. For the Taylor extension to be applicable, the imaginary concentration grid must have the same composition as the grid next to the boundary. Thus, $D_{\varepsilon,1} = D_{\varepsilon,3}$ in both (A) and (B).
3.3.3 Periodic Boundary Condition

Sometimes we are interested only in the diffusion-reaction process within the bulk of the tissue and want to eliminate the surface effect from the computation. In such cases, we can establish the model on a representative unit element of the entire domain and apply the periodic boundary condition to its boundaries. For a two-dimensional domain, this condition means that the top and bottom boundaries are connected and the left and right boundaries are connected, forming a ring torus or “donut” (Figure 3.3).

The mathematical expression of the periodic boundary condition in a three-dimension domain is

$$\mu_{0,j,k} = \mu_{N_x,j,k} \quad \text{and} \quad \mu_{N_x+1,j,k} = \mu_{1,j,k}$$

(3.22)

As an example, we now describe how this condition can be utilized in the $x$ direction.

For $i = 1$, Eqn. (3.8) becomes

$$a \cdot \mu_{1,j,k}^{m+1} - \left( e_3 \cdot \mu_{m+1,0,j,k}^{m+1} + e_1 \cdot \mu_{2,j,k}^{m+1} + e_4 \cdot \mu_{1,j-1,k}^{m+1} + e_2 \cdot \mu_{1,j+1,k}^{m+1} + e_6 \cdot \mu_{1,j,k-1}^{m+1} + e_5 \cdot \mu_{1,j,k+1}^{m+1} \right) = b_{1,j,k}^{m+1}$$

Applying Eqn.(3.22), we get

$$a \cdot \mu_{1,j,k}^{m+1} - \left( e_3 \cdot \mu_{N_x,j,k}^{m+1} + e_1 \cdot \mu_{2,j,k}^{m+1} + e_4 \cdot \mu_{1,j-1,k}^{m+1} + e_2 \cdot \mu_{1,j+1,k}^{m+1} + e_6 \cdot \mu_{1,j,k-1}^{m+1} + e_5 \cdot \mu_{1,j,k+1}^{m+1} \right) = b_{1,j,k}^{m+1}$$

(3.23)
For $i = N_x$, Eqn. (3.8) becomes

$$a \cdot \mu_{N_x,j,k}^{m+1} - \left( e_3 \cdot \mu_{N_x-1,j,k}^{m+1} + e_1 \cdot \mu_{N_x+1,j,k}^{m+1} + e_4 \cdot \mu_{N_x,j-1,k}^{m+1} + e_2 \cdot \mu_{N_x,j+1,k}^{m+1} + e_6 \cdot \mu_{N_x,j,k-1}^{m+1} + e_5 \cdot \mu_{N_x,j,k+1}^{m+1} \right) = b_{N_x,j,k}^{m+1}$$

Applying Eqn. (3.20), we get

$$a \cdot \mu_{N_x,j,k}^{m+1} - \left( e_3 \cdot \mu_{N_x-1,j,k}^{m+1} + e_1 \cdot \mu_{N_x,j,k}^{m+1} + e_4 \cdot \mu_{N_x,j-1,k}^{m+1} + e_2 \cdot \mu_{N_x,j+1,k}^{m+1} + e_6 \cdot \mu_{N_x,j,k-1}^{m+1} + e_5 \cdot \mu_{N_x,j,k+1}^{m+1} \right) = b_{N_x,j,k}^{m+1} \quad (3.24)$$
Figure 3.3: The application of periodic boundary conditions to a two-dimensional domain converts it into a ring torus or “donut.” The two pink lines are used as reference lines for a better understanding of the conversion.
3.4 Solving Discretized PDE's

3.4.1 The Generalized Minimum Residual Method

Equations like Eqn. (3.9) are usually called Poisson equations. They can be solved by basic iterative methods (e.g. Jacobi, Gauss-Seidel and SOR), the Fast Fourier Transform based method\cite{131}, or Krylov subspace methods\cite{132}. For the diffusion-reaction PDE's in our model, the coefficient matrix $A$ can be either symmetric (if all surfaces have Dirichlet boundary conditions) or non-symmetric (if any of the surfaces have mixed or periodic boundary conditions). It is also possible for $A$ to be nearly positive-definite if $|a|$ is very close to $|e_i|$ ($i = 1, 2, \ldots, 6$) in Eqn. (3.8). The Generalized Minimum Residual (GMRES) method, one of the Krylov subspace methods, can overcome these difficulties and can promise, for most cases, fast convergence and high accuracy\cite{133}.

The GMRES method is one of the most important iterative methods available for solving large linear systems in the form of $Ax = b$. It is based on an orthogonal projection onto Krylov subspaces, which are subspaces spanned by vectors of the form $p(A)v$ where $p$ is a polynomial. The basic algorithm of the GMRES method is as follows\cite{133}:

1. Compute $r_0 = b - Ax_0$, $\beta := ||r_0||_2$, and $v_1 := r_0 / \beta$
2. Define the $(m + 1) \times m$ matrix $H_m = \{h_{ij}\}_{1 \leq i \leq m+1, 1 \leq j \leq m}$. Set $H_m = 0$.
3. FOR $j = 1, 2, \ldots, m$ DO:
4. Compute $w_j := Av_j$
5. FOR $i = 1, \ldots, j$ DO:
(6). \[ h_{ij} := (w_j, v_i) \]
(7). \[ w_j := w_j - h_{ij}v_i \]
(8). \[ \text{ENDFOR} \]
(9). \[ h_{j+1,j} = \|w_j\|_2. \text{ If } h_{j+1,j} = 0, \text{ set } m := j \text{ and go to 12} \]
(10). \[ v_{j+1} = w_j / h_{j+1,j} \]
(11). \[ \text{ENDFOR} \]
(12). Compute \( y_m \), the minimizer of \( \|\beta e_1 - H_n y\|_2 \) and \( x_m = x_0 + V_m y_m \).

\subsection{3.4.2 The Preconditioning Technique}

The GMRES method is often used together with a certain preconditioning technique. Roughly speaking, a preconditioner is any form of implicit or explicit modification of an original linear system which makes it “easier” to solve by a given iterative method\(^{[133]}\). For example, scaling all rows of a linear system to make the diagonal elements equal to one is an explicit form of preconditioning. The resulting system can be solved by a Krylov subspace method such as GMRES and may require fewer steps to converge than the original system requires (although this is not guaranteed). As another example, solving the linear system

\[ M^{-1}Ax = M^{-1}b \]

where \( M^{-1} \) is some complicated mapping that may involve Finite Fourier Transforms, integral calculations, and subsidiary linear system solutions, may be another form of preconditioning. Here, it is unlikely that the matrix
$M$ or $M^{-1}$ can be computed explicitly. Instead, the iterative processes operate with $A$ and with $M^{-1}$ whenever needed. In practice, the preconditioning operation $M^{-1}$ should be inexpensive to apply to an arbitrary vector.

One of the simplest ways of defining a preconditioner is to perform an incomplete factorization of the original matrix $A$. This entails a decomposition of the form $A = LU - R$, where $L$ and $U$ have the same nonzero structure as the lower and upper parts of $A$, respectively, and $R$ is the residual or error of the factorization. This incomplete factorization known as ILU(0) is rather easy and inexpensive to compute. On the other hand, it often leads to a crude approximation which may result in the Krylov subspace accelerator requiring many iterations to converge. To remedy this, several alternative incomplete factorizations have been developed by allowing more fill-in in $L$ and $U$. In general, the more accurate ILU factorizations require fewer iterations to converge, but the preprocessing cost to compute the factors is higher. However, if only because of the improved robustness, these trade-offs generally favor the more accurate factorizations. This is especially true when several systems with the same matrix must be solved because the preprocessing cost can be amortized.

### 3.4.3 A Parallel Preconditioned GMRES Solver

As described in Chapter 2, our CA model is implemented on parallel computers. It is thus much desirable to also parallelize the solving of the diffusion-reaction PDE's. Otherwise, the benefit gained from parallelization of the CA part would be lost due to the bottleneck at the step of sequentially
solving the PDE’s. Moreover, iterative methods such as GMRES are ideal candidates for implementation on parallel computers because they only require a rather small set of computational kernels than sparse direct methods do\textsuperscript{[133]}. Writing the code for a parallel GMRES solver, however, can still be very time-consuming for people without degrees in computational science. Fortunately, there are parallel GMRES solvers available. One of the most popular software package of such solvers is called the Portable, Extensible Toolkit for Scientific Computation (PETSc)\textsuperscript{[134]}. Developed by Argonne National Lab, this PDE toolkit provides many preconditioned Krylov subspace methods including GMRES. Compared to other available PDE toolkits, PETSc has the following advantages:

- It is object-oriented and is thus easy to learn. Users are shielded from the details of parallel data communication related to various supported objects, including matrices, vectors, preconditioners and Krylov subspace solvers. Moreover, complete documentation is available in which the usage of almost every routine is illustrated with specific examples.

- It employs existing high-performance packages such BLAS and LAPACK for operations on vectors and matrices. It is thus highly efficient and has proven to be so in many projects similar to ours.

- It provides many of the mechanisms needed within parallel application codes, such as simple parallel matrix and vector assembly routines that allow the overlap of communication and computation.
- It has intensive error checking and debugging functions. Automatic profiling of floating point and memory usage is also readily available.
- It is supported and will be actively enhanced for the next several years. The developers also provide excellent technical support that is fast and detail-oriented.
- It is free.

3.4.4 Algorithm

The algorithm of solving the diffusion-reaction PDE’s is described below. We should note that because the CA time step is usually too large for the PDE solver, it is often evenly divided into smaller PDE time steps and the linear system Eqn. (3.9) is solved repeatedly at each CA iteration in order to achieve acceptable accuracy while maintaining stability.

Before the CA iterations:

(1). Create the following PETSc objects:
   Matrix:  \( A \) (the coefficient matrix)
   Vector:  \( b \) (the right-hand-side vector)
            \( \mu_1 \) (the concentration vector at time step \( m \))
            \( \mu_2 \) (the concentration vector at time step \( m-1 \))
            \( R_1 \) (the cell-uptake vector at time step \( m \))
            \( R_2 \) (the cell-uptake vector at time step \( m-1 \))
   KSP: \( ksp \) (the Krylov subspace solver)

(2). Apply the initial conditions to \( \mu_1 \) and \( \mu_2 \). If any of the surfaces have Dirichlet boundary conditions, apply them, too.
**At the beginning of each CA iteration:**

1. Calculate the effective nutrient diffusivity \((De_x, De_y, De_z)\) between every two concentration points according to local cell density.

2. According to \(De_x, De_y, De_z\), and the boundary conditions, fill non-zero values to the appropriate positions in the coefficient matrix \(A\). With \(A\), set the operator for \(ksp\) and choose the preconditioner and the convergence standard.

3. Calculate the vector \(R_1\). If it is the first time \(R_1\) is calculated, copy \(R_1\) to \(R_2\).

4. With \(De_x, De_y, De_z, \mu_1, \mu_2, R_1\) and \(R_2\), calculate the right-hand-side vector \(b\).

5. Copy \(\mu_1\) to \(\mu_2\) and \(R_1\) to \(R_2\). Save \(\mu_2\) and \(R_2\) to be used in the next CA iteration.

6. With \(ksp\) and \(b\), solve for the concentration vector \(x\) for the next time step. Transfer values in \(x\) to \(\mu_1\) which will be used to modulate cell activities in subsequent routines.

### 3.5 Correctness Check

To check the correctness of the above algorithm, we use our program to solve a diffusion-reaction problem that has an analytical solution and then compare the numerical and analytical solutions. For this purpose, we try to determine the transient concentration profile in a cubic tissue whose side
length is \( L \). The diffusion-reaction PDE with its initial and boundary conditions are shown below:

\[
\frac{\partial C}{\partial t} = D_e \left[ \frac{\partial^2 C}{\partial x^2} + \frac{\partial^2 C}{\partial y^2} + \frac{\partial^2 C}{\partial z^2} \right] - K_{uptake} \cdot C
\]  
(3.25)

Initial condition: \( C = C_i \) for \( x \in (0, L), y \in (0, L), z \in (0, L) \)

Boundary condition: \( C = C_s \) on the six surfaces

where \( D_e \) is the effective diffusivity which is assumed to be constant, and \( K_{uptake} \) is the rate constant of the cell-uptake term. To nondimensionalize Eqn. (3.25), we introduce the following dimensionless variables:

\[
\theta = \frac{C - C_s}{C_i - C_s}, \quad \tau = \frac{D_e \cdot t}{L^2}, \quad \bar{x} = \frac{x}{L}, \quad \bar{y} = \frac{y}{L}, \quad \bar{z} = \frac{z}{L}
\]

\[
\phi^2 = \frac{L^2 \cdot K_{uptake}}{D_e}, \quad k_2 = \phi^2 \cdot \frac{C_s}{C_i - C_s}
\]

Eqn. (3.25) then becomes

\[
\frac{\partial \theta}{\partial \tau} = \left[ \frac{\partial^2 \theta}{\partial \bar{x}^2} + \frac{\partial^2 \theta}{\partial \bar{y}^2} + \frac{\partial^2 \theta}{\partial \bar{z}^2} \right] - \phi^2 \cdot \theta - k_2
\]  
(3.26)

Initial condition: \( \theta = 1 \) for \( \bar{x} \in (0, 1), \bar{y} \in (0, 1), \bar{z} \in (0, 1) \)
Boundary condition: \( \theta = 0 \) on the six surfaces

Eqn. (3.26) with the boundary and initial conditions can be solved with Finite Fourier Transforms and the detail is shown in Appendix B. The final solution is

\[
\theta(x, y, z, \tau) = \sum_{n=1,2} \sum_{m=1,2} \sum_{p=1,2} \left\{ \frac{64}{\pi^3 nmp} \sin(n\pi x) \sin(m\pi y) \sin(p\pi z) \right\} \left[ \left( 1 - \frac{k_2}{\Theta_{nmp}} \right) \exp \left(-\Theta_{nmp} \tau \right) + \frac{k_2}{\Theta_{nmp}} \right]
\]

(3.27)

where \( \Theta_{nmp} = n^2 \pi^2 + m^2 \pi^2 + p^2 \pi^2 + \phi^2 \). The notation \( \sum_{j=1,2}^\infty \) means summation from 1 to \( \infty \) in steps of 2. That is, terms involving \( j = 1, 3, 5, \ldots \) are summed.

We then solve Eqn. (3.26) with our program for the numerical solution and compare it to the solution given by Eqn. (3.27). The solutions are recorded slice by slice into two two-dimensional matrices: \( C_{\text{analy}} \) for the analytical solution and \( C_{\text{numer}} \) for the numerical solution. The relative error of the numerical solution is calculated as

\[
\text{Relative Error} = \frac{\| C_{\text{analy}} - C_{\text{numer}} \|_2}{\| C_{\text{analy}} \|_2} \times 100\%
\]

(3.28)

where \( \| A \|_2 \) is the two norm of a matrix \( A = [a_{ij}] \) where \( i = 1, 2, \ldots, m \) and \( j = 1, 2, \ldots, n \):
\[ \|A\|_2 = \sqrt{\sum_{i=1}^{m} \sum_{j=1}^{n} |a_{i,j}|^2} \]

A series of parametric studies are carried to investigate the effect of model parameters – including the space step size, the time step size, and the cell-uptake rate constant – on the relative error, and the results are plotted in Figure 3.4 – Figure 3.6. Let us estimate what the relative error would be for a realistic case. The diameter of human lung fibroblasts in a tissue is about 10 μm. If we let the characteristic length \( L = 1 \text{ mm} \), \( d\bar{x} \), \( d\bar{y} \) and \( d\bar{z} \) will be 1.0×10⁻². The effective diffusion coefficient of glucose in a variety of biomaterial scaffolds is about 2.7×10⁻¹⁰ m²/s\(^{135-138}\) and the rate constant of glucose consumption by human lung fibroblasts is about 1.0×10⁻⁴ s⁻¹. \( k_1 \) is thus about 0.4. Because the time step for the CA part of the model is usually 0.1 hr, \( d\tau \) will be about 0.1 if the diffusion-reaction PDE is solved only once for each CA time step, causing a relative error of more than 10% in the concentrations. To reduce the relative error, we can divide each CA time step into many smaller PDE time steps. According to the results shown in Figure 3.4 – Figure 3.6, the relative error will be about 0.1% if the PDE is solved 100 times at each CA time step (\( d\tau = 1.0\times10^{-4} \)), an acceptable accuracy for the purpose of our simulations. Since the relative error is affected by the values of space step size, time step size and the cell-uptake rate constant, the guidance provided by Figure 3.4 – Figure 3.6 should be relied on to carefully choose appropriate values of the characteristic length,
characteristic effective diffusivity and PDE time step size so that the relative error remains acceptable.
Figure 3.4: The effect of space step size ($d\bar{x} = d\bar{y} = d\bar{z}$) on the relative error of the numerical solution. Conditions are $d\tau = 1.0 \times 10^{-4}$ and $k_1 = 1.0 \times 10^{-2}$. 
Figure 3.5: The effect of time step size $d\tau$ on the relative error of the numerical solution. Conditions are $d\bar{x} = d\bar{y} = d\bar{z} = 1.0 \times 10^{-2}$ and $k_1 = 1.0 \times 10^{-2}$. 
Figure 3.6: The effect of cell-uptake rate constant $k_1$ on the relative error of the numerical solution. Conditions are $\bar{d}x = \bar{d}y = \bar{d}z = 1.0 \times 10^{-2}$ and $d\tau = 1.0 \times 10^{-4}$. 
CHAPTER 4 MODULATING CELLULAR FUNCTIONS WITH NUTRIENT CONCENTRATION

4.1 Modulating Cell Proliferation

4.1.1 Kinetics

For a cell to grow and replicate, it must not only be in a physical environment with appropriate conditions (e.g., temperature and pH), but it must also have enough nutrients for cellular building and to derive energy for cell functioning. The nutrients required for mammalian cell culture include carbohydrates, amino acids, lipids, fatty acids, vitamins, salts, and trace elements\textsuperscript{[139]}. Among them, oxygen, glucose and glutamine are the most important because of their high demand by the cells. For both oxygen and glucose, the approximate range of uptake rate by mouse hybridoma cells has been reported to be $0.053-0.59\times10^{-9}$ mmol/(cell-hr)\textsuperscript{[140]}. Glutamine is utilized 3-4 times more slowly than glucose is, but 5-10 times faster than any other consumed amino acids\textsuperscript{[141-143]}.

The effect of nutrients on cell proliferation is usually quantified by the cell population doubling rate $r_g$, which in the exponential growth phase is defined as
\[ r_g = \frac{\log_2 N_C(t) - \log_2 N_0}{t} \]  

(4.1)

where \( N_0 \) is the initial cell number and \( N_C(t) \) is the cell number at time \( t \). Various kinetic equations have been developed to relate nutrient concentration \( C_N \) to \( r_g \). The commonly used ones are the modified Contois kinetics\[^{144}\]:

\[ r_g = \frac{r_{g,\text{max}} C_N^n}{K_c C_{\text{cell,overall}} + C_N^n} \]  

(4.2)

and the Moser kinetics\[^{145}\]:

\[ r_g = \frac{r_{g,\text{max}} C_N^n}{K + C_N^n} \]  

(4.3)

where \( C_{\text{cell,overall}} \) is the overall cell density, \( r_{g,\text{max}} \) is the maximum cell population doubling rate, and \( K_c \) and \( K \) are the saturation constants. For the modified Contois growth kinetics, the rate of growth is dependent upon the overall cell density \( C_{\text{cell,overall}} \). This type of kinetic function represents inhibition of growth due to “over-population” of the system. The Moser growth rate is analogous to cooperative enzyme kinetics\[^{146}\] where the nutrient concentration is raised to the \( n \)-th power. When the power is equal to one, we obtain the most commonly used Monod\[^{147}\] growth kinetics:
\[ r_g = \frac{r_{g,\text{max}} C_N}{K + C_N} \] (4.4)

Reported values of \( K \) for human lung fibroblasts are \( 2.7 \times 10^{-5} \) M for glucose and \( 1.1 \times 10^{-5} \) M for glutamine\(^{[148]}\).

### 4.1.2 Measurement of Kinetic Parameters

Here we describe the experimental procedure with which \( r_{g,\text{max}} \) and \( K \) can be measured to characterize the effect of glucose concentration on the proliferation of a type of human dermal fibroblasts.

**Materials and Methods**

Human dermal fibroblasts (catalog# CC-0210, Clonetics, CA) are used for this study. The cell culture medium is based on 1X liquid DMEM 11966 (Invitrogen, CA) supplemented with 10\% Fetal Bovine Serum (Sigma-Aldrich, MO) and 1\% Penicillin-Streptomycin (Life Technologies, MD). DMEM 11966 contains L-glutamine, but no glucose or sodium pyruvate. Different amount of D-glucose (Sigma-Aldrich, MO) is added to the basal medium to achieve a series of media with different final glucose concentrations \( C_{glu} \) (see Table 2). \( C_{glu} \) is measured using an HPLC system (Shimadzu Scientific Instruments, Columbia, MD) in Professor Ka-Yiu San’s lab at Rice University.

At Day 1, 1.0 ml cell suspension at the density of \( 1 \times 10^4 \) cells/ml in medium #1 is added into 24-well polystyrene tissue culture plates (Corning,
MA. A total of 240 wells are seeded with the cells (3 wells per medium formula per day for 8 days). The cells are allowed 4 hours to attach to the bottom of the wells. The spent medium is then aspirated and the wells are rinsed with PBS. Subsequently, medium #2 – #11 are added to the designated wells. Every day from Day 1 to Day 8, cells in 3 wells of each medium condition are lifted from the bottom of wells with Trypsin-EDTA (Life Technologies, MD) and counted using a Coulter Multisizer (Beckman Coulter, CA). Media are replenished on Day 1, Day 3 and Day 6.

Table 2. Cell culture media with different glucose concentrations

<table>
<thead>
<tr>
<th>Medium</th>
<th>$C_{glu}$ (mg/ml)</th>
<th>DMEM 11966 (ml)</th>
<th>D-glucose (mg)</th>
<th>FBS (ml)</th>
<th>PSF (ml)</th>
<th>HPLC measured $C_{glu}$ (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>225.0</td>
<td>0</td>
<td>25</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.09</td>
<td>200.0</td>
<td>18.0</td>
<td>22.2</td>
<td>2.2</td>
<td>0.10593408</td>
</tr>
<tr>
<td>3</td>
<td>0.18</td>
<td>200.0</td>
<td>36.0</td>
<td>22.2</td>
<td>2.2</td>
<td>0.20610304</td>
</tr>
<tr>
<td>4</td>
<td>0.36</td>
<td>200.0</td>
<td>72.0</td>
<td>22.2</td>
<td>2.2</td>
<td>0.4075219</td>
</tr>
<tr>
<td>5</td>
<td>0.54</td>
<td>200.0</td>
<td>108.0</td>
<td>22.2</td>
<td>2.2</td>
<td>0.54354272</td>
</tr>
<tr>
<td>6</td>
<td>0.72</td>
<td>200.0</td>
<td>144.0</td>
<td>22.2</td>
<td>2.2</td>
<td>0.78387616</td>
</tr>
<tr>
<td>7</td>
<td>1.0</td>
<td>200.0</td>
<td>200.0</td>
<td>22.2</td>
<td>2.2</td>
<td>0.91683424</td>
</tr>
<tr>
<td>8</td>
<td>4.0</td>
<td>200.0</td>
<td>600.0</td>
<td>22.2</td>
<td>2.2</td>
<td>2.84778912</td>
</tr>
<tr>
<td>9</td>
<td>4.0</td>
<td>200.0</td>
<td>800.0</td>
<td>22.2</td>
<td>2.2</td>
<td>4.72678976</td>
</tr>
<tr>
<td>10</td>
<td>5.0</td>
<td>200.0</td>
<td>1000.0</td>
<td>22.2</td>
<td>2.2</td>
<td>5.71503552</td>
</tr>
<tr>
<td>11</td>
<td>6.0</td>
<td>200.0</td>
<td>1200.0</td>
<td>22.2</td>
<td>2.2</td>
<td>6.2488496</td>
</tr>
</tbody>
</table>
Results

The cell population doubling rate $r_g$ is calculated from the data of cell numbers using Eqn. (4.1) and is then plotted against glucose concentration $C_{glu}$ in Figure 4.1. The result confirms the applicability of the Mond kinetics, with $r_g$ asymptotically approaching a maximum value when $C_{glu}$ increases. $r_{g,\text{max}}$ and $K$ can be calculated by fitting the experimental data to a transformed Eqn. (4.4):

$$C_{glu} \cdot r_{g,\text{max}} + (-r_g) \cdot K = r_g \cdot C_{glu}$$  \hspace{1cm} (4.5)

We consider $r_{g,\text{max}}$ and $K$ as unknown parameters whose values need to be determined so that, ideally, all the measured values of $C_{glu}$ and $r_g$ can satisfy Eqn. (4.5). With 2 unknowns and 10 data points, however, this is an over-determined linear system as shown by Eqn (4.6) and will have to be solved with the least-square method:

$$
\begin{bmatrix}
C_{glu}^1 & -r_g^1 \\
C_{glu}^2 & -r_g^2 \\
\vdots & \vdots \\
C_{glu}^{10} & -r_g^{10}
\end{bmatrix}
\begin{bmatrix}
k_g \\
K
\end{bmatrix}
=
\begin{bmatrix}
r_g^1 \cdot C_{glu}^1 \\
r_g^2 \cdot C_{glu}^2 \\
\vdots \\
r_g^{10} \cdot C_{glu}^{10}
\end{bmatrix}
$$  \hspace{1cm} (4.6)

where $C_{glu}^i$ and $r_g^i$ ($i = 1, 2, \ldots, 10$) are the $i$-th pair of measured $C_{glu}$ and $r_g$. Using Matlab, which solves over-determined problems with the least-square method, we get $r_{g,\text{max}} = 2.03$ doublings/day and $K = 1.085 \times 10^{-1}$ mg/ml = $6.022 \times 10^{-4}$ M.
Figure 4.1: Experimental results showing the effect of glucose concentration on the population doubling rate $r_g$ of a type of human dermal fibroblasts.
4.1.3 Implementation

When the effect of nutrients on cell proliferation is not considered, cell division time in our discrete CA model is controlled by giving the division counter $Z_{div}$ an initial value calculated according to the following equation and then decrementing $Z_{div}$ by one at each time step:

$$ Z_{div} = \text{int} \left[ \frac{t_d}{\Delta t} \right] \quad (4.7) $$

When cell proliferation is affected by nutrient concentration, however, we need to control the step size at which the division counter is decremented. For this purpose, we define a step size $\Delta Z_{div}$ for the decrement of $Z_{div}$. When the cell is supplied with enough nutrients, $\Delta Z_{div}$ will be equal to its maximum value $\Delta Z_{div,\text{max}}$. Thus, the initial value of $\Delta Z_{div}$ should be calculated as follows so that the cell will divide at a rate equal to $r_{g,\text{max}}$ when there is enough nutrient:

$$ t_{d,\text{min}} = \frac{1}{r_{g,\text{max}}} \quad \text{and} \quad Z_{div} = \Delta Z_{div,\text{max}} \cdot \text{int} \left[ \frac{t_{d,\text{min}}}{\Delta t} \right] \quad (4.8) $$

When the cell is depleted of the nutrient, $\Delta Z_{div}$ should reflect the effect of nutrient concentration $C_N$ on the rate of the division counter’s decrement according to the Monod kinetics:
$$\Delta Z_{\text{div}} = \text{int} \left[ \frac{\Delta Z_{\text{div, max}}}{K + C_N} \cdot \frac{C_N}{K + C_N} \right]$$  \hspace{1cm} (4.9)$$

where $K$ is the saturation constant in Eqn. (4.4). Thus, $\Delta Z_{\text{div}}$ can take any integer value between 0 and $\Delta Z_{\text{div, max}}$, depending on $C_N$. Obviously, the step size can be more accurately calculated according to $C_N$ when $\Delta Z_{\text{div, max}}$ is larger, which results in more precisely modulated cell growth.

To verify the effectiveness of this control mechanism, we run simulations with the model parameters listed in Table 3 and $C_N$ varying from 0.2 mM to 20 mM (invariant in space and in time). If the above control mechanism is effective, the values of $r_{g,\text{max}}$ and $K$ calculated from the simulation results should be the same as those given in Table 3. Following is how this experiment is done:

(7). Calculate $\log_2[\kappa(t)/\kappa_0]$ from the cell volume fraction data shown in Figure 4.2 and plot $\log_2[\kappa(t)/\kappa_0]$ against $t$ for each $C_N$ value (Figure 4.3).

(8). Calculate the slope of the linear part of each curve in Figure 4.3. The value of the slope is equal to the doubling rate $r_g$ of the population under $C_N$.

(9). From Eqn. (4.4), we have

$$\frac{1}{r_g} = \frac{K}{r_{g,\text{max}} C_N} + \frac{1}{r_{g,\text{max}}}$$

Therefore, if $1/r_g$ is plotted against $1/C_N$, we should get a straight line (Figure 4.4) whose slope is equal to $K/r_{g,\text{max}}$ and whose y-axis
intersection is equal to $1/r_{g,\text{max}}$. From Figure 4.4, we obtain $K/r_{g,\text{max}} = 0.3079$ and $1/r_{g,\text{max}} = 0.4918$. These values then produce $r_{g,\text{max}} \approx 2$ doublings/day and $K \approx 0.626$ mM, values very close to the input values for $r_{g,\text{max}}$ and $K$.

The above experiment confirms that by modulating individual cells’ growth rate according to Eqn. (4.9), we can accurately control the collective proliferation rate of the entire population so that it follows the Monod kinetics.

Table 3 The model parameters for the simulation results shown in Figure 4.2.

<table>
<thead>
<tr>
<th>Value</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>100×100×100</td>
<td>Size of the cellular grid</td>
</tr>
<tr>
<td>12</td>
<td>Average cell division time, hr</td>
</tr>
<tr>
<td>50</td>
<td>Cell migration speed, $\mu$m/hr</td>
</tr>
<tr>
<td>2.0</td>
<td>Persistence time in any directional states, hr</td>
</tr>
<tr>
<td>1.0</td>
<td>Aggregation time after a cell-cell collision, hr</td>
</tr>
<tr>
<td>Uniform</td>
<td>Transition probabilities</td>
</tr>
<tr>
<td>6.022D-1</td>
<td>The saturation constant in the Monod kinetics, mole/m$^3$</td>
</tr>
<tr>
<td>100</td>
<td>Maximum step size for division counter</td>
</tr>
</tbody>
</table>
Figure 4.2: The effect of $C_N$ on tissue growth. In these simulations, $C_N$ is uniform throughout the tissue and does not change with time. The other model parameters are the same as in Table 3.
Figure 4.3: Calculating cell population doubling rates from data shown in Figure 4.2. Only linear parts of the curves are shown.
Figure 4.4: The plot of $1/r_g$ against $1/C_N$ for the calculation of $r_{g,max}$ and $K$ in the Monod kinetics.
Having verified the validity of our method of controlling the cell growth rate, we can then implement it in the model. The pseudo-code is described below:

IF the division counter is not zero THEN
   The cell is not ready to divide yet.
   Calculate $\Delta Z_{\text{div}}$ using Eqn. (4.9).
   Decrease the division counter by $\Delta Z_{\text{div}}$.
ELSE
   IF the cell is not completely surrounded THEN
      The cell divides into two daughter cells.
      One of the daughter cells stays at the current site.
      The other daughter cell occupies one of the empty neighboring sites randomly selected.
   ELSE
      The cell cannot divide.
      Assign the cell a new division counter randomly selected from the PDF of the division time.
   ENDIF
ENDIF

4.2 Modulating Cell Migration

4.2.1 Kinetics

As mentioned in Chapter 2, directional movement against the concentration gradient of a certain substance (e.g., a growth factor) is called chemotaxis\textsuperscript{[118-121]}. While many nutrients have been found chemotactic to
bacteria\textsuperscript{[149-153]}, such a phenomenon has not been observed on mammalian cells such as fibroblasts and endothelial cells. Thus, it is assumed in our model that nutrient concentration has no effect on the directional preference of cell migration. On the other hand, nutrients are the energy source for cell function and are needed to maintain cell motility. Kouvrkouglo et al.\textsuperscript{[85]} found that the migration of a highly metastatic prostate cancer cell line slowed down significantly when cells were moved from glucose-containing to glucose-free medium. The quantitative relation between nutrient concentration $C_N$ and cell migration speed $S$, however, has not been established. In our model, the following assumption is made:

\[
\begin{align*}
\text{If } C_N &\leq C_{N,\text{min}}, \\
\text{If } C_N &< C_{N,\text{min}} < C_{N,\text{max}}, \\
\text{If } C_N &\geq C_{N,\text{max}},
\end{align*}
\]

\[
S = \begin{cases} 
0, & \text{if } C_N \leq C_{N,\text{min}}, \\
S_{\text{max}} \frac{(C_N - C_{N,\text{min}})}{(C_{N,\text{max}} - C_{N,\text{min}})}, & \text{if } C_{N,\text{min}} < C_N < C_{N,\text{max}}, \\
S_{\text{max}}, & \text{if } C_N \geq C_{N,\text{max}}.
\end{cases}
\] (4.10)

where $C_{N,\text{min}}$ is the threshold nutrient concentration below which cell migration stops and $C_{N,\text{max}}$ is the critical nutrient concentration above which cells migrate at the maximum speed $S_{\text{max}}$.

\subsection{Implementation}

The following pseudo-code describes how the modulation of cell migration is implemented in our model:
IF $C_N < C_{N, \text{min}}$ THEN
   The cell cannot move or change its direction.
   It enters the stationary state in which the movement counter stops.
ELSE
   IF the movement counter is not zero THEN
      The cell is not ready to move yet.
      Calculate $\Delta Z_{\text{mov}}$ using Eqn (4.10).
      Decrease the movement counter by $\Delta Z_{\text{mov}}$.
ELSE
   The cell is ready to move.
   IF the persistence counter is not zero THEN
      Move the cell to the next position in the current direction.
      Assign the cell a new movement counter.
ELSE
   The cell is ready to make a turn.
   Randomly select the next direction towards one of the empty neighboring sites.
   Move the cell to the selected site.
   Assign the cell a new movement counter and a new persistence counter.
ENDIF
ENDIF
ENDIF
CHAPTER 5  TRANSPORT LIMITATIONS AND TISSUE GROWTH

5.1  The Hybrid Tissue Growth Model

Chapters 2 through 4 discussed the development of the three major components of our model. In this Chapter, these components will be combined to build a hybrid model that can simulate tissue growth under diffusional limitations. This model will be tested and used to design optimal perfusion reactors that can counteract the adverse effects of transport limitations on tissue growth. We will use glucose as an example of vital nutrients for this study.

5.1.1  Discrete Model for Cell Migration and Proliferation

The model developed in Chapter 2 will be used to describe the processes of migration, proliferation and cell-cell collision in the scaffold. We will assume that the average distance between two cells in the scaffold is 20 μm. Thus, the side of each cubic computational site in the cellular grid is \( h = 20 \) μm. A cellular grid with size equal to 100×100×100 sites will correspond to a cubic scaffold with side equal to \( L = 2 \) mm. The cell locomotory and proliferative parameters for the base case are given in Table 4.
5.1.2 The Diffusion-Reaction PDE

The PDE describing the diffusion and reaction of glucose in the tissue system is similar to Eqn. (3.5):

$$\frac{\partial C}{\partial t} = \nabla \cdot (D_e \cdot \nabla C) - \rho_{cell}(x, y, z, t) \frac{V_{max} \cdot C}{K_m + C} \quad (5.1)$$

where $C$ is glucose concentration, $D_e$ is the effective diffusion coefficient, $V_{max}$ and $K_m$ are parameters in the Michaelis-Menten kinetics of glucose uptake by cells, and $\rho_{cell}(x, y, z, t)$ is the local cell density at the point $(x, y, z)$ at time $t$. We ignore the natural degradation term because it is very small for glucose.

The initial condition for Eqn. (5.1) is

$$C(x, y, z, 0) = 0 \quad (5.2)$$

This corresponds to the situation in which a seeded scaffold is just emerged into a bioreactor filled with cell culture medium. Depending on the configuration of the bioreactor and the tissue culture conditions, Dirichlet, Neumann, or mixed boundary conditions described by Eqn. (3.3) can be applied to the surfaces of the scaffold.

If there is a perfusion channel in the scaffold, the mixed boundary condition Eqn. (3.12) is applied to the channel wall:
\[ D_e \nabla_n (C) \big|_{w} = k_g (C_b - C_w) \]  

(5.3)

where \( k_g \) is the mass transfer coefficient from the medium flow to the tissue, \( C_b \) is glucose concentration in the bulk of the medium and \( C_w \) is glucose concentration at the channel wall. We assume that the medium flow rate is high enough for the axial convection to be ignored.

Eqn. (5.1) with the given initial and boundary conditions can be nondimensionalized, discretized and solved following the procedure described in Chapter 3. Realistic values of the involved parameters are obtained from previous studies in the literature:(5.1)

**Effective Diffusion Coefficient**

In the discrete cellular automaton, a computational site can be empty or occupied (thought not completely filled) by a cell. The effective glucose diffusivity obviously depends on local cell density. We use \( D_{e,s} \) and \( D_{e,t} \) to denote the diffusivity in cell-free and fully-occupied regions, respectively. Bringi and Dale\(^{136}\) measured \( D_{e,s} \) for glucose in porous compressed glass fiber mat supports fabricated into disks. The average value was \( 3.0 \times 10^{-10} \) m\(^2\)/s. Alexandre et al.\(^{135}\) found that \( D_{e,s} \) was about \( 2.5 \times 10^{-10} \) m\(^2\)/s for glucose through acellular pure poly(ethylene oxide) (PEO) star hydrogels or PEO star hydrogel modified with expanded polytetrafluoroethylene membranes. Leddy et al.\(^{137}\) measured \( D_{e,s} \) for 4 types of dextran in bioartificial cartilages made from agarose, alginate, fibrin and gelatin. Extrapolation from their data for blank fibrin scaffold gives a value of \( 2.7 \times 10^{-10} \) m\(^2\)/s for \( D_{e,s} \). A similar value was found by Li et al.\(^{138}\) for glucose diffusion in a type of collagen-copolymer corneal implant. Thus, we assume...
that $D_{e,s}$ is $2.7 \times 10^{-10}$ m$^2$/s for cell-free regions in the scaffold. $D_{e,t}$ is obviously lower than $D_{e,s}$. Its value in natural stroma is about $7.0 \times 10^{-11}$ m$^2$/s$^{[45]}$ and about $8.0 \times 10^{-11}$ m$^2$/s in articular cartilage$^{[46]}$. We use the value $7.0 \times 10^{-11}$ m$^2$/s for $D_{e,t}$ in our model. When discretizing Eqn.(5.1), we will need to calculate the diffusivity for grid points that are between an empty site and an occupied site. For such points, the average diffusivity is calculated as

$$D_{e,avg} = \frac{2 \cdot D_{e,s} \cdot D_{e,t}}{D_{e,s} + D_{e,t}}$$  \hspace{1cm} (5.4)

**Cell Density**

The cell density $\rho_{cell}(x, y, z, t)$ in Eqn (5.1) is a discontinuous function of the occupancy of site $(x, y, z)$ at time $t$:

$$\rho_{cell}(x, y, z, t) = \begin{cases} 
0 & \text{if site } (x, y, z) \text{ is empty at time } t. \\
\frac{1}{h^3} & \text{if site } (x, y, z) \text{ is occupied at time } t.
\end{cases}$$

By the above rule, we are assuming that the consumption of glucose by the cell in a computational site will only affect the mass balance within in that site.

**Michaelis-Menten Kinetic Parameters**

As mentioned in Chapter 4, Fleischaker et al.$^{[140]}$ found that the approximate range of glucose uptake rate by mouse hybridoma cells was
$0.53 - 5.9 \times 10^{-13}$ mol/(cell·h). For isolated rat lung cells, Pérez-Díaz et al.\textsuperscript{[154]} reported that $V_{\text{max}}$ and $K_m$ were $3.31 \times 10^{-13}$ mol/(cells·hr) and 2.4 mol/m$^3$, respectively. Thus, we will use $V_{\text{max}} = 3.31 \times 10^{-13}$ mol/(cells·hr) and $K_m = 2.4$ mol/m$^3$ in our model.

**Mass Transfer Coefficient**

As a very dilute aqueous solution, cell culture medium can be considered to have approximately the same viscosity ($\mu$), density ($\rho$) and glucose diffusivity ($D$) as those of water. We can then use these values (see Table 4) to calculate the mass transfer coefficient from the medium flow to the wall of perfusion channels.

The medium flow rate $Q$ in perfusion systems is usually 0.1-1.0 ml/min for a tissue 10 mm in diameter and 2 mm in thickness\textsuperscript{[49, 61]}. If one perfusion channel is created in the scaffold and its diameter ranges from 1 mm to 8 mm, the Reynolds number $Re$ is between 3.8 and 60 if medium flow rate is 1 ml/min. This indicates laminar flow behavior. For such cases, the average value of Sherwood number $Sh$ over the entire tissue thickness $x$ should be calculated as\textsuperscript{[155-157]}:

$$
Sh = 3.66 + \frac{0.0668 \frac{d}{x} \cdot Re \cdot Sc}{1 + 0.04 \left[ \frac{d}{x} \cdot Re \cdot Sc \right]^2^{\frac{2}{3}}} \quad (5.5)
$$
where \( d \) is the diameter of the perfusion channel, channel diameter and \( Sc (= \frac{\mu \cdot D}{\rho}) \) is the Schmidt number. Once \( Sh \) is known, \( k_g \) can be calculated as

\[
k_g = \frac{Sh \cdot D}{d}
\]  
(5.6)

We should note here that the \( k_g \) calculated with Eqn. (5.5) and Eqn. (5.6) is the average of the mass transfer coefficient over the perfusion channel whose length is equal to the thickness \( x \) of the tissue.

### 5.1.3 Modulation of Cell Functions

For the modulation of cell growth, the saturation constant \( K \) in Eqn. (4.4) is \( 6.022 \times 10^{-1} \) mole/m\(^3\) and the maximum step size \( \Delta Z_{div, \text{max}} \) in Eqn. (4.9) is 100. For the modulation of cell migration speed, \( C_{N,\text{min}} \) and \( C_{N,\text{max}} \) in Eqn. (4.10) are 0.0 mole/m\(^3\) and 5.0 mole/m\(^3\), respectively. The value 5.0 mole/m\(^3\) is the concentration of glucose in human serum\(^{158}\). We assume that cells migrate at full speed when local glucose concentration is equal to or higher than this value.

### 5.1.4 Simulation Procedure

The above model components are combined to form a hybrid model whose algorithm is depicted by the flow chart in Figure 5.1. After the initial seeding of cells, the sites occupied by perfusion channels (if any) are located and marked as the “channel sites” so that cells cannot move in. The state of
the channel sites remains constant throughout the simulation. The glucose concentration at such sites is also assumed to be constant.

Before the CA iterations start, the PETSc objects needed for solving the nondimensionalized diffusion-reaction PDE are defined and initialized (see detail in Chapter 3). Within each CA iteration, the linear system Eqn. (3.9) is first solved repeatedly at a PDE time step size small enough for the GMRES solver to maintain both accuracy and stability. After all the PDE iterations are done, the newly computed concentrations are imported into subsequent CA routines to modulate cell migration, growth and division. The CA iterations continue until the end of the designated simulation time is reached.
Figure 5.1: The flow chart showing the algorithm of the hybrid tissue growth model.
5.2 Simulating Tissue Growth without Perfusion Channels

With the above hybrid model, we first study tissue growth without the perfusion channels. The purpose is to simplify the problem for a better understanding of the involved mass transfer dynamics. Here we focus on the system described by Figure 5.2 in which several seeded cubic scaffolds are fixed on needles and are cultured in a well-stirred bioreactor. We assume that the mixing of the tissue culture medium is so well that the glucose concentration on scaffold surfaces is the same as in the bulk of the medium. Thus, the Dirichlet boundary conditions Eqn. (3.10) can be applied to the scaffold surfaces. We also assume that the initial cell volume fraction is $\kappa_0 = 0.01$ and the tissues will be cultured for 10 days in this bioreactor.

To identify the factors that may lead to severe mass transfer limitation during the tissue growth process, we recall the Thiele modulus introduced in Chapter 3:

$$\phi^2 = \frac{\rho_{cell} V_{\text{max}} (L)^2}{D_e C_s}$$

(5.7)

where $C_s$ is the glucose concentration on scaffold surfaces and the meaning of the other parameters are the same as in Eqn. (5.1). According to Eqn. (5.7), the following conditions will cause severe mass transfer limitation inside the scaffold:

- High cell density
- High glucose consumption rate by cells
- Large tissue size
- Low glucose diffusivity
- Low glucose concentration on scaffold surface

The highest possible cell density in our model does not change because the side length $h$ of the computation sites is fixed. Thus, we will quantitatively study the effect of the other four parameters on mass transfer dynamics and, consequently, tissue growth. For this purpose, we define a base case whose model parameters are listed in Table 4. We should note that due to the limitation imposed by the available computing resources, it is impossible for us to run simulations for tissues a few centimeters in side length (a cubic 2 cm$^3$ tissue, for example, would require a 1000×1000×1000 cellular grid). The mass transfer limitation in smaller tissues, however, will be insignificant. As a compromise, we use a 100×100×100 cellular grid and reduce the values of both $D_{e,s}$ and $D_{e,i}$ to 1% of their realistic values (see Table 4). According to Eqn. (5.7), the mass transfer limitation for this configuration will be equivalent to that in a 1000×1000×1000 cellular grid with the original values of $D_{e,s}$ and $D_{e,i}$. This can also save us a large amount of CPU time needed to run the simulations described next.

Figure 5.3 shows the temporal evolution of the cell volume fraction $\kappa(t)$ for the base case. After a short lag phase in the beginning, $\kappa(t)$ increases rapidly between $t = 2.0$ days and $t = 5.0$ days. The growth rate then starts to decrease and finally reaches a plateau with $\kappa(t)$ increasing only by 0.071 from Day 5 to Day 10. The pattern of tissue growth and the distribution of
glucose concentration are shown by images in Figure 2.4 and Figure 5.10, respectively. $\bar{C}_{\text{min}}$ in Figure 5.10 is the normalized minimum glucose concentration in the scaffold: $\bar{C}_{\text{min}} = C_{\text{min}}/C_s$. We can see that the concentration front penetrates slightly into the scaffold at the beginning of the simulation, but then quickly recedes towards the surface. In most of the scaffold interior, the concentration stays very low throughout the simulation. As a result, cells can only grow within a thin layer next to the scaffold surface (Figure 2.4). It seems that the layer of cells creates a bottleneck effect which prevents glucose concentration in the scaffold interior from rising high enough to support normal cell proliferation. It is therefore very clear that growing a bioartificial tissue of the given size ($2^3 \text{ cm}$) will be very difficult under the tissue culture conditions shown in Figure 5.2.

Next, we will change the values of the parameters in Eqn. (5.7) to see how they affect the above dynamic process.
Figure 5.2: Seeded bioartificial tissues cultured in a well-stirred bioreactor.
Table 4. The base-case model parameters for simulating the growth of three-dimensional tissues

<table>
<thead>
<tr>
<th>Value</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>100×100×100</td>
<td>Size of the cellular grid</td>
</tr>
<tr>
<td>0.2</td>
<td>Time step for the CA model, hr</td>
</tr>
<tr>
<td>10</td>
<td>Tissue culture time, days</td>
</tr>
</tbody>
</table>

**Parameters for cells**

- 20: Cell diameter, μm
- 12: Average cell division time, hr
- 2: Variance in the normal distribution of cell division time
- 25: Cell migration speed, μm/hr
- 2.0: Persistence time in directional states 0, 1, 2, …, and 6, hr
- Uniform: Transition probabilities
- 1.0: Aggregation time after cell-cell collisions, hr

**Parameters for the diffusion-reaction PDE**

- 2.7E-12: Glucose diffusivity in empty scaffold, m²/s
- 7.0E-13: Glucose diffusivity in filled scaffold, m²/s
- 2.5: Glucose concentration on scaffold surfaces, mole/m³
- 3.31×10⁻¹³: Glucose consumption rate constant, mol/(cells·hr)
- 2.4: The saturation constant for glucose consumption rate, mole/m³

**Parameters for the perfusion channel (if there is one)**

- 9.1E-10: Glucose diffusivity in the medium, m²/s
- 1.0: Medium flow rate through the perfusion channel, ml/min
- 6.92E-4: Medium viscosity, kg/(m·s)
- 993.25: Medium density, kg/m³
Figure 5.3: The temporal evolution of cell volume fraction $\kappa(t)$ for the growth of a typical three-dimensional bioartificial tissue described in Figure 5.2. Model parameters for this simulation run are listed in Table 4.
Figure 5.4: The pattern of tissue growth in the three-dimensional scaffold for the base case whose tissue growth curve is shown in Figure 5.3.
Figure 5.5: The distribution of the normalized glucose concentration in the three-dimensional scaffold for the base case whose tissue growth curve is shown in Figure 5.3.
Effect of Tissue Size

The first parameter we will study is the size of the tissue which is characterized by the side length $L$. The legend $\lambda_L$ in Figure 5.6 is defined as $\lambda_L = L/L^*$, where $L^*$ is the value of the side length in Table 4. Figure 5.6 shows that as $L$ increases, it takes longer for the tissue growth curve to reach the plateau. The effect of $\lambda_L$ on the final value of $\kappa(t)$, $\kappa_{final}$, is described by Figure 5.7.

Effect of Glucose Consumption Rate

In Figure 5.8, tissue growth curves are plotted for increasing values of $V_{max}$. The legend $\lambda_{V_{max}}$ is defined as $\lambda_{V_{max}} = V_{max}/V^*_{max}$, where $V^*_{max}$ is the value of glucose consumption rate in Table 4. We can see that as cells' demand on glucose lowers, the tissue grows increasingly faster to reach higher values of $\kappa_{final}$, confirming the prediction of Eqn. (5.7). To see the pattern of tissue growth and the distribution of glucose concentration, images similar to those shown in Figure 2.4 and Figure 5.10 are generated in Figure 5.4 and Figure 5.5 respectively for the case $\lambda_{V_{max}} = 0.01$ in Figure 5.8. Because of the low glucose consumption rate, $\overline{C}_{min}$ in the scaffold has already reached 0.8816 after 2.0 days (Figure 5.10B). As the cells proliferate in the scaffold, however, the concentration fronts slowly recedes towards the scaffold surface. By $t = 6.0$ days, $\overline{C}_{min}$ is almost zero in most of the scaffold interior (Figure 5.10D) and the tissue has almost reached confluence. The effect of $\lambda_{V_{max}}$ on $\kappa_{final}$ is described by the plot in Figure 5.11.
Effect of Glucose Diffusivities

In Figure 5.12, the diffusivity of glucose in the scaffold and the tissue are decreased simultaneously to the fraction $\lambda_{De}$ (shown in the legend of Figure 5.12) of their respective values in Table 4. That is,

$$\lambda_{De} = \frac{D_{e,\text{scaffold}}}{D_{e,\text{scaffold}}^*} = \frac{D_{e,\text{tissue}}}{D_{e,\text{tissue}}^*}$$

where $D_{e,\text{scaffold}}^*$ and $D_{e,\text{tissue}}^*$ are the values of the diffusivities in Table 4. It seems that the decrease in glucose diffusivity has a similar effect as when the glucose consumption rate increases. There is, however, a noticeable difference. While all the curves in Figure 5.8 reach the plateau at about the same time, curves with higher values of $\lambda_{De}$ in Figure 5.12 reach the plateau earlier. This is because higher $\lambda_{De}$ means the diffusion of glucose is more efficient and, thus, the balance between diffusion and reaction can be reached sooner. The effect of $\lambda_{De}$ on $\kappa_{\text{final}}$ is described by the plot in Figure 5.13.

Effect of Surface Concentration

The glucose concentration on scaffold surfaces is characterized by the ratio $\lambda_{Cs} = C_s / C_s^*$, where $C_s^*$ is the value of surface concentration in Table 4. Figure 5.14 shows that higher surface concentration has a similar effect as higher glucose diffusivity, with the tissue reaching the plateau earlier at a higher final value.
The above parametric studies show that our simulation results are in accordance with the predictions of the Thiele modulus. In addition, the pattern of tissue growth precisely reflects the distribution of glucose concentration inside the scaffold. To further demonstrate our model's ability of simulating tissue growth under different culture conditions, we have run a simulation in which all the model parameters are the same as in the case "\( \lambda_{v_{\text{max}}} = 0.01 \)" in Figure 5.8 except that the bottom surface of the scaffold has the Neumann boundary condition. This corresponds to the situation in which the seeded scaffold is cultured in a Petri dish and its bottom surface is isolated from the bulk of the medium. Figure 5.15 shows the tissue growth curves for these two conditions. The difference is not very significant, mainly because the mass transfer limitation is not very severe with the given model parameters. The glucose concentration can rise quickly inside the scaffold even if the bottom surface is isolated from the medium. The pattern of tissue growth and the distribution of glucose concentration, however, are quite different as shown by Figure 5.16 and Figure 5.17. The lack of concentration gradient in the direction towards the bottom surface (Figure 5.17) and the pattern of remaining unfilled regions in the scaffold (Figure 5.16D) both reflect the difference made by the zero-flux condition applied to the bottom surface.
Figure 5.6: The effect of tissue size (characterized by $\lambda_L$ whose value is shown in the legend) on the temporal evolution of cell volume fraction $\kappa(t)$. The other model parameters are the same as in Table 4.
Figure 5.7: The effect of tissue size (characterized by $\lambda_L$) on the final cell volume fraction $\kappa_{final}$ in the three-dimensional scaffold. The other model parameters are the same as in Table 4.
Figure 5.8: The effect of glucose consumption rate (characterized by $\lambda_{V_{\text{max}}}$ whose value is shown in the legend) on the temporal evolution of cell volume fraction $\kappa(t)$. The other model parameters are the same as in Table 4.
Figure 5.9: The pattern of tissue growth in the three-dimensional scaffold for the case \( \lambda_{v_{\text{max}}} = 0.01 \) in Figure 5.8.
Figure 5.10: The distribution of the normalized glucose concentration in the three-dimensional scaffold for the case $\lambda_{V_{\text{max}}} = 0.01$ in Figure 5.8.
Figure 5.11: The effect of glucose consumption rate (characterized by $\lambda_{V_{\text{max}}}$) on the final cell volume fraction $\kappa_{\text{final}}$ in the three-dimensional scaffold. The other model parameters are the same as in Table 4.
Figure 5.12: The effect of glucose diffusivities (characterized by $\lambda_{De}$ whose value is shown in the legend) on the temporal evolution of cell volume fraction $\kappa(t)$. The other model parameters are the same as in Table 4.
Figure 5.13: The effect of glucose diffusivities (characterized by $\lambda_{De}$) on the final cell volume fraction $\kappa_{final}$ in the three-dimensional scaffold. The other model parameters are the same as in Table 4.
Figure 5.14: The effect of glucose concentration of scaffold surface (characterized by $\lambda_{cs}$ whose value is shown in the legend) on the temporal evolution of cell volume fraction $\kappa(t)$. The other model parameters are the same as in Table 4.
Figure 5.15: The effect of boundary conditions on the temporal evolution of cell volume fraction $\kappa(t)$. The case "Uniform Dirichlet" is the same as the case $\lambda_{v_{max}} = 0.01$ in Figure 5.8. The only difference between the two curves in this figure is that the Neumann boundary condition is applied to the bottom surface of the scaffold for "Bottom Neumann".
Figure 5.16: The pattern of tissue growth in the three-dimensional scaffold for the case “Bottom Neumann” in Figure 5.15.
Figure 5.17: The distribution of the normalized glucose concentration in the three-dimensional scaffold for the case “Bottom Neumann” in Figure 5.15.
5.3 Tissue Growth with Perfusion Channels

The images in Figure 5.4 and Figure 5.5 indicate that tissue growth is limited to regions close to the scaffold surfaces when significant mass transfer limitation appears. Thus, the idea of creating perfusion channels in the scaffold has been logically proposed to reduce the distance of cells from sources of high glucose concentration\cite{70}. We have run a test simulation to confirm the possible benefit of this idea. For the base case whose tissue growth curve is shown in Figure 5.3, we create a perfusion channel in the center of the scaffold. The diameter $d$ of the channel satisfies $d/L = 0.2$. We assume that the medium flow rate through the channel is high enough for the glucose concentration to be considered constant throughout the entire channel. Using the parameters given in Table 4, we then calculate the mass transfer coefficient from the bulk of the medium flow to the channel wall. We should note that the diffusivity of glucose in the medium is also reduced by 100 times for the same scaling reason discussed at the beginning of this section.

Figure 5.18 shows the tissue growth curves before and after the perfusion channel is created. In the beginning, the two curves almost overlap each other. After about 2.5 days, however, their difference becomes increasingly larger with $\kappa(t)$ being higher for the perfused tissue. The final cell volume fraction $\kappa_{\text{final}}$ is 0.3230 for the case without the perfusion channel and 0.3712 for the other. To see how the presence of the perfusion channel affects the pattern of tissue growth and the distribution of glucose concentration, images similar to those in Figure 5.4 and Figure 5.5 are
created for the perfused tissue in Figure 5.19 and Figure 5.20, respectively. We can see that the perfusion channel brings a ring-shaped high glucose concentration zone in the center of the scaffold where cells can proliferate. Although the concentration front in that zone also quickly recedes towards the channel, the overall cell volume fraction is still increased by about 15%. Obviously, the size of the channel will affect the concentration profile in the scaffold and, consequently, growth of the tissue. The question then is “How big should this channel be?” A channel too small will be inadequate in overcoming the mass transfer limitation. On the other hand, an unnecessarily large channel will waste valuable scaffold space without which cells cannot grow. It may also jeopardize the mechanical integrity of the scaffold. Things become more interesting when we consider the possibility of having multiple channels in the scaffold. Will that benefit the tissue growth process and if yes, how should we arrange the channels to achieve the optimal outcome? All these questions constitute a design problem that will be solved with our hybrid model in the rest of this chapter.
Figure 5.18: The effect of the presence of a perfusion channel on tissue growth. The curve named “No Perfusion” is for the base case shown in Figure 5.3. The “With Perfusion” curve is for the growth of the same tissue with a perfusion channel in the center. The other model parameters are the same as in Table 4.
Figure 5.19: The pattern of tissue growth in the three-dimensional scaffold for the case “With Perfusion” in Figure 5.18.
Figure 5.20: The distribution of the normalized glucose concentration in the three-dimensional scaffold for the case “With Perfusion” in Figure 5.18.
5.3.1 The Design Problem

The perfusion channels can have various shapes (e.g., circular and square) and can be arranged in many different patterns. Here we will focus on circular channels arranged in the regular square pattern shown in Figure 4.1. There are then two design parameters to be determined: the channel diameter $d$ and the channel pitch $l$ (Figure 4.1). The channel pitch is the distance between two perfusion channels and is thus an indication of cells’ distance from sources of high glucose concentration. It will certainly have an effect on the mass transfer dynamics in the tissue. In order to study this parameter, we need to be able to change the size of the cellular grid over a wide range. If a three-dimensional cellular grid is used, however, the grid size we can experiment on will be seriously limited by the available computational resources. To relax this constraint, we define the domain for our optimization problem to be a representative slice (one layer of cells in thickness) in the tissue that is perpendicular to the direction of the medium flow (Figure 4.1). This will reduce the dimension of the problem from three to two and, thus, give us much greater freedom in choosing the grid sizes. In the previously mentioned study of Kofidis et al.\cite{Kofidis}, very small variations were found in cell density along the direction of the medium flow in perfused bioartificial cardiac tissues. This indicates that the nutrient concentration gradient exists mainly in the direction perpendicular to the perfusion channels. Of course, this conclusion will not hold for very thick tissues. For the tissues that we are studying, we think it is acceptable to
assume that the rate and patter of tissue growth on the representative slice can reflect how the entire tissue is growing.

If the representative slice is very large and there are many perfusion channels in it, the mass transfer through scaffold surfaces will become relatively insignificant compared to that through the channels. Under such conditions, we can exploit the symmetry in the pattern of perfusion channels and further reduce the domain for the optimization problem to a representative unit element (RUE) in the representative slice (Figure 4.1). To eliminate the surface effect on the simulation results, we apply the periodic boundary conditions on the periphery of the RUE for both the CA model and the diffusion-reaction PDE. This means the two-dimensional domain will form a ring torus (see Figure 3.3). Therefore, when cells leave the cellular grid from one side, it will immediately enter the cellular grid at the exact opposite site on the other side of the cellular grid. If that site is empty, the cell will occupy it. Otherwise, it will collide with the cell occupying that site (Figure 5.22). Since the perfusion channel in the RUE may have different sizes, it should be emphasized that the definition of the cell volume fraction $\kappa(t)$ is the following:

$$\kappa(t) = \frac{N_{C}(t)}{L^2}$$  
(5.8)

where $N_{C}(t)$ is the number of cells in the tissue at time $t$. Note that the volume of the entire RUE, instead of only the volume of useable scaffold space, is used in the calculation of $\kappa(t)$. This is reasonable because when
evaluating a design, we are interested in how many cells can be harvested in the entire scaffold, including the space occupied by perfusion channels. Figure 5.23 shows that one may have a completely filled tissue with a large channel, but its overall cell volume fraction can still be lower than that of a partially filled tissue with a smaller channel.

In summary, the optimization problem we will solve is to search for the combination of \( d \) and \( l \) for the RUE shown in Figure 4.1 so that the cell volume fraction \( \kappa(t) \) defined by Eqn. (5.8) is the highest at the end of a given tissue culture period. To be consistent with the simulations we have completed so far, the tissue culture period will still be 10 days.
Figure 5.21: The simplification of the domain on which the optimization problem is defined.
Figure 5.22: The effect of applying periodic boundary conditions on the periphery of the cellular grid. Blank sites are empty and pink sites are occupied by cells. The cell leaving at site 1 will immediately enter the cellular grid at site 2. The cell leaving at site 3 will immediately collide with the cell at site 4.
Figure 5.23: A higher cell volume fraction in the usable scaffold space does not necessarily mean the overall cell volume fraction is also higher.
5.3.2 Results

To solve the optimization problem described above, we define a base case in which the channel pitch \( l = 8 \) mm and the channel diameter \( d = 2 \) mm. Since the simulations will now run on large two-dimensional cellular grids, we will be able to use the realistic values for all model parameters which are listed in Table 5. Those whose values are different from in Table 4 are highlighted with bold font style. The tissue growth curve for the base case is shown in Figure 5.24. The pattern of tissue growth and the distribution of glucose concentration in the scaffold are shown in Figure 5.25 and Figure 5.26, respectively. Like what we have seen before, tissue growth here is also limited by the distance that glucose can reach into the scaffold interior. Next, we will carry out a series of parametric studies in which the effect of channel diameter, channel pitch, glucose consumption rate and glucose diffusivity on \( \kappa_{\text{final}} \) will be investigated.

First, we fix the value of \( l \) and see how \( \kappa_{\text{final}} \) will change if we vary \( d \). Figure 5.27 contains several growth curves for the values of \( d \) varying between 1 mm and 7 mm. We can see that curves with smaller values of \( d \) start to rise earlier. This is due to two reasons: (a) scaffolds with smaller channels have more cells left after the channel is created, and (b) the mass transfer coefficient \( k_s \) is affected reversely by the value of \( d \) (Figure 5.28). As the simulation continues, however, these advantages gradually diminish and can be completely lost before the simulation ends (see, for example, the curves for \( d = 4 \) mm and \( d = 1 \) mm). This is because as cells proliferate in the scaffold and their demand on the supply of glucose increases, the gain in
the initial number of cells and the value of \( k_g \) can be overweighed by the loss in the area of channel-scaffold interface through which mass transfer can take place. That is not to say, however, that a larger \( d \) is always more desirable. Figure 5.27 shows that \( \kappa_{\text{final}} \) reaches a maximum when \( d = 5 \) mm and then decreases with increasing \( d \). From the plot of the final volume fractions of cells, the channel and the empty space against \( d \) in Figure 5.29, we can see clearly that there exists an optimal channel diameter \( d_{\text{opt}} \) at which \( \kappa_{\text{final}} \) is the highest (We name this value \( \kappa_{\text{final,opt}} \). A \( d \) that is too large (e.g., \( d = 7 \) mm) should not be chosen because it results in the loss of too much usable scaffold space and a value of \( k_g \) that is too low.

The value of \( d_{\text{opt}} \) in Figure 5.29 is for the base case in which the channel pitch \( l \) is fixed at 8 mm. What will happen if we change the value of \( l \)? How will the values of \( d_{\text{opt}} \) and \( \kappa_{\text{final,opt}} \) shift? These are what we will find out next. For values of \( l \) ranging from 4 mm to 12 mm, a series of simulations like those shown in Figure 5.27 are carried out. The results of \( d \) and \( \kappa_{\text{final}} \) are plotted in Figure 5.30. We can see that \( d_{\text{opt}} \) increases with increasing \( l \). This is understandable because a larger \( l \) means perfusion channels are farther apart and according to the Thiele modulus in Eqn. (5.7), the mass transfer limitation will become severer. Thus, larger channels are needed to achieve the optimal \( \kappa_{\text{final}} \). Figure 5.30 also indicates that \( \kappa_{\text{final,opt}} \) drops as \( l \) increases. To gain more insight into this phenomenon, the final volume fractions of the perfusion channel and the empty space in the scaffold for the optimal case \( (d = d_{\text{opt}}) \) are plotted against the volume of the scaffold \( (l^2) \) in Figure 5.31 and Figure 5.32, respectively. These plots reveal that when the channel pitch increases, (a) a higher fraction of the scaffold
space has to be sacrificed as the perfusion channel in order to achieve $\kappa_{\text{final, opt}}$, and (b) even if the optimal channel diameter is used, a higher fraction of the scaffold space will remain empty at the end of the tissue culture period. It is always more desirable, therefore, to have a smaller channel pitch. In reality, however, there is usually a technical limit on how dense the channels can be created in the scaffold. The mechanical integrity of the tissue is another limiting factor. With results shown in Figure 5.30, we can tell what the optimal channel diameter would be for the smallest possible channel pitch.

Having understood how $d$ and $l$ should be chosen for a system in which all the other parameters are fixed, we then investigate how the changes in glucose consumption rate $V_{\text{max}}$ and the glucose diffusivity in tissue $D_{e,t}$ may affect the values of optimal $d$ and $l$.

**Effect of Glucose consumption Rate**

The effect of $V_{\text{max}}$ on $d_{\text{opt}}$ and $\kappa_{\text{final}}$ is shown in Figure 5.33. We can see that $d_{\text{opt}}$ increases with increasing $V_{\text{max}}$. This is because cells with higher $V_{\text{max}}$ consume glucose faster and larger channels are therefore needed to achieve the optimal $\kappa_{\text{final}}$. The decreasing value of $\kappa_{\text{final, opt}}$ indicates that fewer cells can possibly be harvested when $V_{\text{max}}$ increases.

**Effect of Nutrient Diffusivity in Tissue**

The effect of decreasing $D_{e,t}$ on $d_{\text{opt}}$ and $\kappa_{\text{final}}$ is very similar to that of increasing $V_{\text{max}}$ (Figure 5.34). Indeed, the magnitude of these two parameters, characterizing respectively the nutrient’s diffusion and reaction
properties, should be evaluated against each other. For example, a $V_{max}$ that is too high for one system may not be so for another system where $D_{e,t}$ is much higher. This is because a nutrient diffusing very easily in the scaffold can keep its concentration from dropping too low even when its consumption rate is high.
Table 5. The base-case model parameters for the design problem of perfusion channels.

<table>
<thead>
<tr>
<th>Value</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>400×400</td>
<td>Size of the cellular grid</td>
</tr>
<tr>
<td>0.2</td>
<td>Time step for the CA model, hr</td>
</tr>
<tr>
<td>10</td>
<td>Tissue culture time, days</td>
</tr>
</tbody>
</table>

**Parameters for cells**

<table>
<thead>
<tr>
<th>Value</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>Cell diameter, μm</td>
</tr>
<tr>
<td>12</td>
<td>Average cell division time, hr</td>
</tr>
<tr>
<td>2</td>
<td>Variance in the normal distribution of cell division time</td>
</tr>
<tr>
<td>25</td>
<td>Free cell migration speed, μm/hr</td>
</tr>
<tr>
<td>2.0</td>
<td>Persistence time in directional states 0, 1, 2, …, and 6, hr</td>
</tr>
<tr>
<td>Uniform</td>
<td>Transition probabilities</td>
</tr>
<tr>
<td>1.0</td>
<td>Aggregation time after cell-cell collisions, hr</td>
</tr>
</tbody>
</table>

**Parameters for the diffusion-reaction PDE**

<table>
<thead>
<tr>
<th>Value</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.7E-10</td>
<td>Glucose diffusivity in empty scaffold, m²/s</td>
</tr>
<tr>
<td>7.0E-11</td>
<td>Glucose diffusivity in filled scaffold, m²/s</td>
</tr>
<tr>
<td>2.5</td>
<td>Glucose concentration on scaffold surfaces, mole/m³</td>
</tr>
<tr>
<td>3.31×10⁻¹³</td>
<td>Glucose consumption rate constant, mol/(cells·hr)</td>
</tr>
<tr>
<td>2.4</td>
<td>The saturation constant for glucose consumption rate, mole/m³</td>
</tr>
</tbody>
</table>

**Parameters for the perfusion channel (if there is one)**

<table>
<thead>
<tr>
<th>Value</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.1E-10</td>
<td>Glucose diffusivity in the medium, m²/s</td>
</tr>
<tr>
<td>1.0</td>
<td>Medium flow rate through the perfusion channel, ml/min</td>
</tr>
<tr>
<td>6.92E-4</td>
<td>Medium viscosity, kg/(m·s)</td>
</tr>
<tr>
<td>993.25</td>
<td>Medium density, kg/m³</td>
</tr>
</tbody>
</table>
Figure 5.24: The temporal evolution of cell volume fraction $\kappa(t)$ for the growth of a typical perfused tissue. Model parameters for this simulation run are listed in Table 5.
Figure 5.25: The pattern of tissue growth in the perfused scaffold whose growth curve is shown in Figure 5.24.
Figure 5.26: The distribution of the normalized glucose concentration in the perfused scaffold whose growth curve is shown in Figure 5.24.
Figure 5.27: The effect of channel diameter $d$ (shown in legend) on the temporal evolution of cell volume fraction $\kappa(t)$. The other model parameters are the same as in Table 5.
Figure 5.28: The effect of channel diameter $d$ on the value of mass transfer coefficient $k_g$ calculated with Eqn. (5.6) using the parameters in Table 5.
Figure 5.29: The effect of channel diameter $d$ on the final volume fractions of cells, the channel, and empty space in the scaffold. The other model parameters are the same as in Table 5.
Figure 5.30: The combined effect of channel pitch $l$ (shown in legend) and channel diameter $d$ on $\kappa_{\text{final}}$. The other model parameters are the same as in Table 5.
Figure 5.31: The effect of scaffold volume on the final channel volume fraction in the scaffold when the optimal channel diameter $d_{opt}$ is chosen. The other model parameters are the same as in Table 5.
Figure 5.32: The effect of scaffold volume on the final volume fraction of empty space in the scaffold when the optimal channel diameter $d_{opt}$ is chosen. The other model parameters are the same as in Table 5.
Figure 5.33: The combined effect of channel diameter $d$ and the glucose consumption rate constant $V_{max}$ (shown in legend) on the final cell volume fraction $\kappa_{final}$. The other model parameters are the same as in Table 5.
Figure 5.34: The combined effect of channel diameter $d$ and the glucose diffusivity in the tissue $D_{e,t}$ (shown in legend) on the final cell volume fraction $\kappa_{final}$. The other model parameters are the same as in Table 5.
5.4 Summary

A comprehensive hybrid three-dimensional model has been developed in this chapter by combining the three major components discussed in the previous chapters. Realistic model parameters are chosen to study the mass transfer dynamics during the growth of three-dimensional tissues. Our results can confirm the predictions of the Thiele modulus. Large tissue sizes, low nutrient diffusivity, high nutrient consumption rates, and low surface concentration are identified as the conditions leading to sever mass transfer limitation. This limitation can be alleviated by adding perfusion channels to the scaffold. The design of such channels, however, constitutes an optimization problem whose solution requires a hybrid computer model like ours. Ignoring the surface effect for a large tissue with many circular perfusion channels, we define the optimization problem on a two-dimensional representative unit element in the tissue and consider the rate and pattern of tissue growth in this element as a reflection of the growth of the entire tissue. This simplification makes it possible for us to study the effect of tissue sizes on the design of perfusion channels. Simulation results for this part show that (a) there exists an optimal channel diameter at which the highest cell volume fraction can be achieved at the end of the tissue culture period, and (b) whenever the mass transfer limitation becomes severer, larger channels will be needed to achieve the optimal final cell volume fraction and the value of it will be decreasing.
CHAPTER 6  SIMULATING ACID-MEDIATED EARLY-STAGE TUMOR GROWTH

6.1  Introduction

The estimated new cases and death of various forms of cancer in the US are 1,368,030 and 563,700 in 2004, respectively\cite{159}. Such prevalence and lethality are the driving forces for the intensive worldwide study on cancer pathology and treatment. Most cancers derive from a single abnormal cell that has undergone a change in its DNA sequence (mutation)\cite{160}. If the mutation enables cells to proliferate in defiance of normal restraints and invade and colonize territories normally reserved for other cells, a cancer results. Depending on whether or not they can invade the surrounding tissue, tumors are defined as invasive (malignant) and noninvasive (benign). The progression of invasive cancer is often categorized as Stage I, II, III, or IV\cite{161}. Stages I and II are considered the "early stages" in the sense that the tumor has not yet spread to distant parts of the body through bloodstream or lymphatic vessels to form secondary tumors (metastasis). Most mathematical modeling of tumor growth focuses on this stage because that is when the tumor is still well defined and treatments likely to be most effective.

The evolution of any ecological system is the result of competition and natural selection. The growth of an invasive tumor is no exception. In order to invade the surrounding tissue, cancer cells must have competitive
phenotypes enabling them to outgrow, or suppress the growth of, normal cells. Warburg\textsuperscript{[162]} first described in 1930 that cancer cells preferentially convert glucose and other substrate to lactic acid, even in the presence of abundant oxygen. This phenotype, later found to be a consequence of mutation\textsuperscript{[163]}, results in a several fold increase in glucose uptake and lactic acid production in tumor cells versus normal cells. In fact, the increase is so marked that it is now routinely used to diagnose many types of tumors\textsuperscript{[164, 165]} and to monitor early tumor response to therapies\textsuperscript{[166]}. This seemingly less efficient way of utilizing substrate plays an important role in tumor growth. The accumulation of lactic acid can cause the pH at the tumor-host interface to drop to about 0.5 pH units lower than in normal tissues\textsuperscript{[167-170]}. Because tumor cells are much more tolerant of acidic extracellular pH\textsuperscript{[171]}, such a microenvironment facilitates tumor growth by reducing the viability of normal cells, breaking the intercellular gap junctions essential for cohesion, collaboration and communication among normal cells\textsuperscript{[172, 173]}, and stimulating the production of enzymes that degrade the ECM at the tumor edge\textsuperscript{[174-176]}. As normal cells die and decompose, tumor cells can proliferate and take over their position in the tissue (tumor invasion). Described in Figure 6.1, this mechanism of acid-mediated tumor growth (AMTG) has been supported by many recent reports that high lactate levels are directly correlated with the likelihood of metastases, tumor recurrence and restricted patient survival\textsuperscript{[177-181]}.

Of course, cancer cells are not unlimtedly tolerant of acidic environment. Dairkee et al.\textsuperscript{[182]} found that breast cancer cells cultured \textit{in vitro} entered a quiescent state (in which cell division cannot occur) when
extracellular pH was lowered to 6.8 and committed apoptosis when local pH was further lowered to about 6.0. One type of cancer therapy is based on the idea of lowering the pH in cancer cells to a lethal level by blocking the pathway through which intracellular pH is regulated\textsuperscript{183, 184}. Because lactic acid is produced by tumor cells and is removed by blood vessels embedded in the tissue, its concentration in the tumor system (the tumor, the surrounding normal tissue, and the network of blood vessels) is determined by two key factors: tumor cell acid production rate and host vascularity. In order for the tumor to grow efficiently, these two factors must combine to create a microenvironment that is acidic enough to kill normal cells at the tumor edge, but not so acidic as to suppress tumor cells' own activity (autoxicity). For a given tumor cell acid production rate, a host vascularity that is too low will result in the accumulation of excessive amount of acid in the tumor, creating quiescent or even necrotic regions inside the tumor. A host vascularity that is too high is also not favorable because it will prevent the extracellular pH from lowering to an intolerable level for normal cells by rapidly removing the lactic acid. This is likely why tumors often induce the growth of new blood vessels from the host tissue toward themselves, a process called angiogenesis\textsuperscript{40, 41}. Angiogenesis has been found to be a prerequisite for the growth and metastasis of solid tumors\textsuperscript{185-187}.

We believe that a quantitative understanding of the AMTG can help us to better understand the effect of aforementioned key factors on tumor growth and, thereby, design more effective cancer treatment medicines/strategies (e.g., the anti-angiogenic therapies\textsuperscript{188-190}). The complexity of the process necessitates a systematical approach similar to
how the growth of perfused bioartificial tissues was studied in the previous chapters. In fact, the help of predictive computer models is even more important for the study of tumor growth because clinical trials in this area usually involve human subjects and are thus much more expensive and time-consuming than in vitro tissue culture experiments.

![Diagram showing cell growth and labeling]

- Normal cell
- Cancerous cell
- Dying normal cell

Figure 6.1: The schematic showing the acid-mediated tumor growth. The dashed arrowheads indicate the secretion of H⁺ ions by tumor cells.

### 6.2 Previous Work

A two-dimensional continuum reaction-diffusion model for the AMTG was formulated by Gatenby and Gawlinski[191]. The model predicted
the extension of a pH gradient from the tumor-host interface, causing a hypocellular interstitial gap at the propagating tumor-host interface. It also predicted the effect of this pH gradient on the tumor’s crossover from benign to malignant states. This fully deterministic model, however, is incapable of describing each individual cell’s history, which is very important in the early stage when only a small number of tumor cells are present and the state of any of them may affect the entire course of tumor growth. It is therefore only useful for modeling relatively large (more than a few millimeters in diameter), clinically apparent tumors.

As an improvement to the Gatenby and Gawlinski model, Patel et al.\cite{192} proposed a hybrid two-dimensional model to simulate AMTG. This model was based on a cellular automaton that incorporated normal cells, tumor cells, necrotic space, and a random network of parallel native capillaries in the cellular grid. Two diffusion-reaction PDE’s described the convection and diffusion of glucose and H\(^+\) ions in the tumor system as well as their utilization or production by cells. Both normal and cancerous cells could be active, quiescent or dead, depending on local pH. Their simulation results demonstrated that for every acid production rate, there exists a range of optimal capillary densities within which tumor growth is most effective. The model was also able to produce a variety of tumor morphology observed in clinical studies by adjusting the values of key model parameters. There is, however, a major drawback in the Patel et al. model. Being two-dimensional, it is not capable of describing the complex three-dimensional vasculature in tumors, which, as discussed earlier, plays a very important role in regulating tumor growth. This limitation may be the reason for the
following discrepancies between their simulation results and previously reported experimental results or clinical observations:

- Tumor cell acid production rates lower than those observed *in vivo* had to be used in order for the computed pH field to be realistic. (We should note here that this problem may have also resulted because the Patel et al. model did not compute the glucose and H\(^+\) concentrations by solving the diffusion-reaction PDE’s explicitly in time, but rather by solving a sequence of equilibrium, elliptic boundary-value equations on a much coarser time scale. This simplification certainly introduced errors into the concentrations.)

- Tumors were able to grow very large without having necrosis in their interior. This contrasts to clinical observations that necrosis universally exists in malignant tumors larger than several millimeters in diameter\(^{[161, 193-195]}\).

If we consider the fact that blood vessels are essentially the perfusion channels *in vivo*, the basic characters of a tumor system are then very similar to those of a perfused bioartificial tissue. Thus, we consider it feasible to modify our hybrid tissue growth model developed in the previous chapters to simulate the AMTG. With its ability of describing realistic, three-dimensional vasculature in the tumor system, our model may be able to lift, at least to some extent, the above limitations. Our effort is also encouraged by the fact that our model can solve the PDE’s accurately with a highly efficient GMRES solver and has a mechanism of individually modulating cell activities according to local concentrations of important substances.
According to our discussion earlier, both features are very important for simulating tumor growth. In this chapter, we will first address the issue of incorporating three-dimensional capillary networks into our model. We will then describe in detail the automaton rules and the diffusion-reaction PDE’s. Finally, simulation results will be presented and analyzed with the emphasis on their difference from those given by the Petal et al. model.

6.3 Model Description

6.3.1 Three-Dimensional Capillary Networks

Capillaries are the smallest blood vessels that can directly exchange substances with cells. They are typically less than 1 mm long with diameter ranging from 10 to 15 μm\textsuperscript{[196]}. Capillary density varies considerably in the vascular beds of different organs: 2500-3000/mm\textsuperscript{3} in brain, kidney, liver, and myocardium; 300-400/mm\textsuperscript{3} in phasic units of skeletal musculature; and less than 100/mm\textsuperscript{3} in bone, fat, connective tissue, and in tonic units of skeletal musculature\textsuperscript{[37]}. For our study, we need discrete representations of realistic three-dimensional capillary networks with various vascularity \( \phi \), so that we can investigate the effect of \( \phi \) on tumor growth. Secomb et al.\textsuperscript{[197]} provided a set of data representing a small portion of the capillary network in R3230AC mammary carcinoma in rat dorsal skin. The data give the diameter, length and Cartesian coordinates of 104 capillary segments in a 550 μm × 520 μm × 230 μm region (Figure 6.2). The capillary density is
1581/mm$^3$, and $\phi_c$ is 1.43%. Similar data with other values of vascularity, however, are not available. Therefore, we have developed a bifurcating distributive algorithm with which tree-like three-dimensional capillary networks can be generated in computers. The number of generations of bifurcation can be controlled to achieve different overall host vascularity. Although the topology of such computer-generated capillary networks are not exactly the same as that of the real ones, we will show that the simulation results of tumor size and the number of tumor cells are very close if their vascularity is approximately the same. Thus, we consider it a reasonable compromise to use the computer-generated capillary networks in order to study the effect of host vascularity on tumor growth.

6.3.1.1 A Real Three-Dimensional Capillary Network

The histogram of the distribution of segment diameter in the Secomb et al. data is shown in Figure 6.3, and the average is 9.89 $\mu$m. Since our CA model has a uniform cellular grid, it cannot describe the different diameters of the capillary segments. Thus, we assume that every capillary segment is 10 $\mu$m in diameter so that a discrete approximation of the Secomb et al. data can be generated in a $55\times52\times23$ three-dimensional cellular grid in which the side of each cubic computational site is 10 $\mu$m. This assumption also makes it convenient to incorporate cells into the cellular grid because the diameter of fibroblasts in rat skin is approximately 10 $\mu$m$^{[33]}$. A $55\times52\times23$ grid, however, is too small for our plan to simulate tumor growth for at least 20 generations. According to the Patel et al. study, a tumor will be about 0.6
mm in diameter and the acidic region around it will be even larger after that period of time\textsuperscript{[192]}. Of course, our results are likely to be different, but we can still use their results for a rough estimation. Therefore, sixteen of this unit element are stacked together to produce a 110×104×92 three-dimensional capillary network (Figure 6.4). Because the distribution of capillaries is not uniform, the stacking is done as follows to avoid having regions of higher or lower vascularity being clustered together at the interfaces of unit elements:

(1). The basic unit (unit 1) is repeated in $x$ direction: For a node point at $(x_1, y_1, z_1)$, another node point is generated at $(N_x+x_1, y_1, z_1)$.

(2). The newly generated unit (unit 2) is rotated 180 degrees around the axis perpendicular to $y$-$z$ surface at $(N_y/2, N_z/2)$: The node point at $(x_2, y_2, z_2)$ is moved to $(x_2, N_y-y_2, N_z-z_2)$.

(3). Units 1 and 2 now combine to form unit 3 which is then repeated in $y$ direction: For a node point at $(x_3, y_3, z_3)$, another node point is generated at $(x_3, N_y+y_3, z_3)$.

(4). The newly generated unit (unit 4) is rotated 180 degrees around the axis perpendicular to $x$-$z$ surface at $(N_x, N_z/2)$: The node point at $(x_4, y_4, z_4)$ is moved to $(2N_x-x_4, y_4, N_z-z_4)$.

(5). Units 3 and 4 now combine to form unit 5 which is then repeated in $z$ direction: For a node point at $(x_5, y_5, z_5)$, another node point is generated at $(x_5, y_5, N_z+z_5)$.

(6). The newly generated unit (unit 6) is rotated 180 degrees around the axis perpendicular to $x$-$y$ surface at $(N_x, N_y)$: The node point at $(x_6, y_6, z_6)$ is moved to $(2N_x-x_6, 2N_y-y_6, z_6)$.
(7). Units 5 and 6 now combine to form unit 7 which is then repeated in z direction: For a node point at \((x_7, y_7, z_7)\), another node point is generated at \((x_7, y_7, 2N_z + z_7)\).

(8). The newly generated unit (unit 8) is rotated 180 degrees around the axis perpendicular to x-y surface at \((N_x, N_y)\): The node point at \((x_8, y_8, z_8)\) is moved to \((2N_x - x_8, 2N_y - y_8, z_8)\).
Figure 6.2: A portion of the three-dimensional capillary network in R3230AC mammary carcinoma in rat dorsal skin. Data and image provided by Secomb et al[^197].
Figure 6.3: The histogram of the distribution of capillary segment diameter in the data provided by Secomb et al.\cite{197}.
Figure 6.4: A modified and extended three-dimensional capillary network generated from the data provided by Secomb et al.\textsuperscript{[197]}. 
6.3.1.2 Computer-Generated Three-Dimensional Capillary Networks

In normal vasculature, 98% of blood vessels bifurcate at each junction while the rest (2%) trifurcate\textsuperscript{[198-200]}. The branching angle ranges from 25 to 140 degrees\textsuperscript{[201]}. At each branching junction the daughter segments tend to have a smaller diameter and length than the parent segment. Based on such topological information, a bifurcating distributive algorithm was developed by Burrowes et al.\textsuperscript{[202]} to create a geometric mesh of the capillary network over the surface of a single alveolar sac. We add the following assumptions to their algorithm in order to develop an algorithm that is simpler yet still useful for our study:

- The length of capillary segments is constant. Since the average length of segments in the Secomb et al. data is about 71 \(\mu m\), the length of capillary segments is set to be 70 \(\mu m\) in our algorithm.

- The diameter of capillary segments is also constant. It is equal to the average segment diameter (10 \(\mu m\)) in the Secomb et al. data.

- All branches are generated by bifurcations.

- Branching angles must be between 25 and 140 degrees.

The following is the modified bifurcating distributive algorithm used in our model:

(1). The first two branches are randomly generated so that the second branch ends at the center of the mass of the tissue.
(2). The first branch and the center of the mass are used to define a splitting plane. The splitting plane is extended to the tissue surfaces, and the grid points on either side of the plane are assigned to two subcollections.

(3). The center of the mass of each subcollection of grid points is calculated.

(4). An imaginary line is constructed from the end of the previous branch to each center of the mass.

(5). For each subcollection of grid points, a branch is generated from the end of the previous branch, lying on the imaginary line and extending the defined length toward the center of the mass.

(7). The position of the branch end is checked to make sure it is inside the tissue. If the branch end is outside the tissue, its length is reduced until the end point lies within the host. The branch then becomes a terminal capillary.

(8). The number of grid points in the subcollection is checked. If there is only one point, the branch is a terminal capillary.

(9). The process continues until the designated generation number is reached or until all branches are terminated by a terminal capillary.

Figure 6.5 shows a $55 \times 52 \times 23$ three-dimensional capillary network generated using the above algorithm. A total of six generations of segments are produced, and the overall vascularity is 1.41%, very close to that of the real capillary network shown in Figure 6.2. Sixteen of this unit element are then stacked together following the stacking procedure described earlier,
producing a $110 \times 104 \times 92$ three-dimensional capillary network (Figure 6.6). The topology of this computer-generated capillary network is obviously different from the one shown in Figure 6.4, but, as will be shown later, the results of tumor size and the number of tumor cells computed with these two networks are very close.
Figure 6.5: A $55 \times 52 \times 23$ three-dimensional capillary network generated with the bifurcating distributive algorithm. Each segment is $10 \mu m$ in diameter and $70 \mu m$ in length. The overall vascularity is 1.41%.
Figure 6.6: A 110×104×92 three-dimensional capillary network generated by stacking 16 of the unit element shown in Figure 6.5.
6.3.2 The Cellular Automaton

The tumor, the surrounding normal tissue, and the capillary network are modeled as a three-dimensional cellular automaton. Sites in the cellular grid can be empty or occupied by one of the following six types of cells: active normal cells, quiescent normal cells, active tumor cells, quiescent tumor cells, necrotic tumor cells, or capillary cells. Thus, a cell type index is added to the state \( x_i \) for the \( i \)-th site in an \( N_x \times N_y \times N_z \) cellular grid, see Chapter 2) of each site to carry this information. In order to compare our simulation results to the results from the Patel et al. study, the initial conditions and automaton rules for our model are very similar to theirs.

Initialization

The initial location and diameter of the tumor are among the input parameters, and, accordingly, a certain number of sites are occupied by tumor cells when the simulation starts. A discrete representation of the three-dimensional capillary network is then incorporated into the cellular grid. All of the remaining sites are occupied by normal cells. The division counter of normal or tumor cells is initialized according to given probability density functions of division time (see Chapter 2).

Automaton Rules

- The state of capillary cells does not change. Their glucose concentration \( C_G \) and \( H^+ \) concentration \( C_H \) also remain constant.
• Normal and tumor cells do not move but can divide. The step size at which the division counter decreases is modulated by local glucose concentration according to the Monod kinetics (see Chapter 4). The selection of a neighboring site when a cell divides is the same as described in Chapter 4.

• Both normal and tumor cells consume glucose, but only tumor cells secrete lactic acid.

• There are several critical concentrations for normal and tumor cells:
  □ If local $C_G$ is lower than a critical value $C_{G}^{N,D}$ for normal cells and $C_{G}^{T,D}$ for tumor cells, the cell will die and decompose due to hypoglycemia\textsuperscript{[203]}. The site will then become empty.
  □ If local pH is lower than a critical value $\text{pH}_{N}^{Q}$ for normal cells and $\text{pH}_{T}^{Q}$ for tumor cells, the cell will enter a quiescent state in which mitosis cannot occur. Tumor cells in this state produce lactic acid at a rate much lower than when they are active (details will be described later). For quiescent cells, if local pH is higher than the quiescenting value, the cell becomes active again and the decrement of its division counter resumes.
  □ If local pH is further lower than another critical value $\text{pH}_{N}^{D}$ for normal cells and $\text{pH}_{T}^{D}$ for tumor cells, the cell will die and decompose because of the lethally acidic extracellular environment. The site will then become empty.

6.3.3 Diffusion-Reaction PDE’s
Like the Patel et al. model, our model will only consider the diffusion and reaction of glucose and lactic acid. Other important substances such as oxygen are considered abundant and uniformly distributed in the tumor system. The diffusion-reaction PDE for glucose is

$$\frac{\partial C_G}{\partial t} = \nabla \cdot \left[ D_{e,G} \nabla (C_G) \right] - G_U^{(\text{cell type})} \cdot C_G \quad (6.1)$$

where $D_{e,G}$ is glucose diffusivity and $G_U^{(\text{cell type})}$ is glucose uptake rate. The value of $D_{e,G}$ in the Patel et al. study was $9.1 \times 10^{-9}$ m$^2$/s, which, if not a misprint, is very far from the correct value. As mentioned in Chapter 5, glucose diffusivity in natural stroma is about $7.0 \times 10^{-11}$ m$^2$/s$^{[45]}$. Cascari et al.$^{[204]}$ found that glucose diffusivity in multicellular tumor spheroids is in the range of $2.3 \times 10^{-11}$ m$^2$/s to $1.1 \times 10^{-10}$ m$^2$/s. In this study, we set $D_{e,G} = 5.5 \times 10^{-11}$ m$^2$/s. The value of $G_U^{(\text{cell type})}$ depends on cell type:

$$G_U^{(\text{cell type})} = \begin{cases} G_U^N & \text{for active and quiescent normal cells} \\ G_U^T & \text{for active and quiescent tumor cells} \\ 0.0 & \text{for empty or microvessel cells} \end{cases}$$

where $1.0 \times 10^{-6}$ s$^{-1} < G_U^N < 5.0 \times 10^{-5}$ s$^{-1}$ and $1.0 \times 10^{-5}$ s$^{-1} < G_U^T < 1.0 \times 10^{-3}$ s$^{-1}$ $^{[167, 194, 205]}$. Here we set $G_U^N = 1.0 \times 10^{-4}$ s$^{-1}$ and $G_U^T = 1.0 \times 10^{-3}$ s$^{-1}$, values exactly the same as those in the Patel et al. study.

The diffusion-reaction PDE for lactic acid is
\[
\frac{\partial C_H}{\partial t} = \nabla \cdot \left[ D_{e,H} \nabla (C_H) \right] + H_p^{(\text{cell type})} 
\] (6.2)

where \( D_{e,H} \) is the effective diffusivity of lactic acid and \( H_p^{(\text{cell type})} \) is lactic acid production rate. The value of \( D_{e,H} \) used in the Patel et al. study was \( 1.08 \times 10^{-9} \) m\(^2\)/s which is close to what other studies have reported. The value of \( H_p^{(\text{cell type})} \) depends on cell type:

\[
H_p^{(\text{cell type})} = \begin{cases} 
0.0 \text{ mM/s} & \text{for normal, empty or capillary cells} \\
H_p^{T,A} & \text{for active tumor cells} \\
H_p^{T,Q} & \text{for quiescent tumor cells}
\end{cases}
\]

where \( H_p^{T,A} \) and \( H_p^{T,Q} \) are the rates of lactic acid production for active and quiescent tumor cells, respectively. As quiescent tumor cells are essentially metabolically inactive, \( H_p^{T,Q} \) is set to be \( 0.01 H_p^{T,A} \)\(^{[192]}\). Thus, \( H_p^{T,A} \) is a key parameter reflecting different phenotypes of tumor metabolism. In the parametric studies later, we will search for the range of this important parameter necessary for maintaining normal pH field in the tumor system and for facilitating tumor growth. Previous experimental studies have reported its value to be between \( 1.0 \times 10^{-4} \) mM/s and \( 3.0 \times 10^{-4} \) mM/s\(^{[167,205]}\).

6.3.4 Modulation of Cell Proliferation

As mentioned earlier, cell proliferation is controlled by local glucose concentration according to the Monod kinetics:
where \( r_{g,\text{max}} \text{ (cell type)} \) is the maximum cell population doubling rate and \( K_{\text{cell type}} \) is a saturation constant, both of which depend on the cell type. In this study, we assume that \( r_{g,\text{max}}^N = r_{g,\text{max}}^T = 2 \) doublings/day (this means the average cell division time is 12 hr). The saturation constant for normal cells \( K^N \) is set to be 0.6022 mole/m\(^3\) which is the value that we measured for Human Dermal Fibroblasts in Chapter 4. Since it is well known that tumor cells are generally less dependent on nutrient supplies\(^{160}\), the saturation constant for tumor cells \( K^T \) will be smaller than \( K^N \). Its effect on tumor growth will be investigated in the parametric studies later.

### 6.4 Results

Because the goal of this study is to see if our hybrid three-dimensional model can overcome the aforementioned limitations in the Patel et al. model, we will focus on the following two questions when analyzing our simulation results: (a) To maintain a normal pH field and facilitate tumor growth, are the values of \( H^{T,A}_p \) used in our model closer to the experimental values? (b) Is tumor necrosis a universal phenomenon in our simulation results? In addition, we will investigate the effect of tumor quiescenting pH \( pH^Q_T \), host vascularity \( \phi_v \), and the saturation constant \( K^T \) in Eqn. (6.3) on tumor growth.
A series of parametric studies will be carried out for which the base-case model parameters are listed in Table 6. The tumor starts from a spheroid about 80 μm in diameter and all simulations last for 20 generations of cell division. Because the distribution of capillaries is not uniform in the cellular grid, the tumor will not grow into a perfect sphere. Instead, its front will protrude through regions where local vascularity is most favorable. To characterize tumor size, therefore, we define the following radius of gyration $R_T$ for the entire tumor including regions of active tumor cells, quiescent tumor cells and tumor necrosis (if there is any):

$$R_T = \sqrt{\frac{\sum_{i=1}^{N_T} [(x_i - x_0)^2 + (y_i - y_0)^2 + (z_i - z_0)^2]}{N_T}}$$

where $N_T$ is the total number of sites occupied by active, quiescent and necrotic tumor cells, $(x_0, y_0, z_0)$ is the original position of the tumor’s center, and $(x_i, y_i, z_i)$ is the position of the $i$-th site.
Table 6. The base-case model parameters for the parametric studies on tumor growth.

<table>
<thead>
<tr>
<th>Value</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>110×104×92</td>
<td>Size of the three-dimensional cellular grid</td>
</tr>
<tr>
<td>10</td>
<td>Average cell diameter, μm</td>
</tr>
<tr>
<td>Periodic</td>
<td>Type of boundary condition on all surfaces</td>
</tr>
<tr>
<td>0.2</td>
<td>Time step for the CA model, hr</td>
</tr>
</tbody>
</table>

**Parameters for normal cells**

| 12             | Average cell division time, hr                               |
| 2              | Variance in the normal distribution of cell division time    |
| 1.0E-4         | $G_U^N$, 1/s                                                 |
| 2.5            | $C_G^{N,D}$, mole/m³                                        |
| 7.1            | pH$_N^Q$                                                    |
| 6.8            | pH$_N^D$                                                    |
| 6.022E-1       | $K^N$, mole/m³                                               |

**Parameters for tumor cells**

| 12             | Average cell division time, hr                               |
| 2              | Variance in the normal distribution of cell division time    |
| (55, 52, 46)   | Original position of the tumor’s center in the cellular grid |
| 4              | Tumor radius, cells                                          |
| 1.0E-3         | $G_U^T$, 1/s                                                 |
| 1.0E-4         | $H_p^{T,A}$, mole/(m³·s)                                    |
| 1.0E-6         | $H_p^{T,Q}$, mole/(m³·s)                                    |
2.5 \( C_{G}^{T,D} \), mole/m³
6.4 \( pH_{T}^{Q} \)
6.0 \( pH_{T}^{D} \)
6.022E-1 \( K_{T}^{T} \), mole/m³

**Parameters for the diffusion-reaction PDE’s**

5.0 Initial glucose concentration, mole/m³
5.0 Glucose concentration in capillaries, mole/m³
5.5E-11 Glucose diffusivity, m²/s
3.0E-7 Glucose mass transfer coefficient at capillary wall, m/s
3.98E-5 Initial lactic acid concentration, mole/m³
3.98E-5 Lactic acid concentration in capillaries, mole/m³
1.08E-10 Lactic acid diffusivity, m²/s
1.19E-6 Lactic acid mass transfer coefficient at capillary wall, m/s

**Effect of Tumor Cell Acid Production Rate**

Figure 6.7 shows the effect of tumor cell acid production rate \( H_{p}^{T,A} \) on the temporal evolution of the tumor’s radius of gyration \( R_{T} \). While all \( R_{T} \) curves in the Patel et al. study are straight lines with slopes affected by the value of \( H_{p}^{T,A} \), the curves in our study display a two-phase behavior: at the beginning, all curves have the same slope; as the simulation continues, however, they start to deviate from the initial phase at different time points. Interestingly, the slopes are almost the same after the deviating points. This two-phase growth behavior is in accordance with experimental observations.
on the growth of tumors in rodent models and patients [206]. Another
significant difference between our results and the results of the Petal et al.
study is that the values of $H_{p,t}^{T,A}$ required to maintain tumor growth are
significantly higher in our study than in theirs. For the same host vascularity
($\phi_r = 1.43\%$), $H_{p,t}^{T,A}$ is between $9.4 \times 10^{-5}$ mM/s and $2.0 \times 10^{-4}$ mM/s in our
study and between $2.5 \times 10^{-5}$ mM/s and $1.0 \times 10^{-4}$ mM/s in theirs. As a more
specific comparison, $H_{p,t}^{T,A}$ is about $9.5 \times 10^{-5}$ mM/s for the tumor to grow
0.32 mm per generation in our study, but it is only $5.0 \times 10^{-5}$ mM/s for the
same rate of tumor growth in theirs. The fact that our results of $H_{p,t}^{T,A}$ are
closer to the experimental values indicates that the ability of describing a
three-dimensional vasculature is very important in simulating tumor growth.
What is similar in both studies, however, is that the tumor seems to
experience a sharp transition from a benign to a malignant state as $H_{p,t}^{T,A}$
increases through a threshold (about $9.1 \times 10^{-5}$ mM/s in Figure 6.7). We
name this value the transition tumor cell acid production rate $H_{p,\text{trans}}^{T,A}$. This
phenomenon has also been predicted by the study of Gatenby and
Gawlinski [191] using a two-dimensional continuum reaction-diffusion model.

The temporal evolution of the total number of tumor cells $N_T$ and the
fraction $\phi_{\text{necrosis}}$ of necrotic cells in the tumor are shown in Figure 6.8A and
Figure 6.8B, respectively. We notice immediately that necrosis exists in all
the simulation runs. The starting time of its appearance and its fraction in
the tumor are affected by the value of $H_{p,t}^{T,A}$: higher $H_{p,t}^{T,A}$ causes necrosis to
appear earlier and to constitute a larger portion of the entire tumor. These
results confirm clinical observations, but are in contrast to the findings of the
Patel et al. study in which, as mentioned earlier, tumors could grow very large without having necrosis at all.

For a better understanding of the relation between acid diffusion and tumor growth, the temporal evolutions of the cell distribution and the pH field in the tumor system are visualized in Figure 6.9 and Figure 2.4, respectively, for the case $H_{p,1}^{T,A} = 1.0 \times 10^{-4}$ mM/s in Figure 6.7. We can see that by Generation 1, a well-defined hypocellular gap has formed before the advancing tumor front (Figure 6.9). Normal cells surrounding the tumor are constantly dying as the region of pH lower than $pH_{N}^{D}$ (6.8) expands (Figure 2.4). Meanwhile, the pH in the central area of the tumor is also dropping due to the accumulation of lactic acid. At approximately $t = 5.6$ days, necrosis starts to appear in the tumor center (Figure 6.8B) and when the simulation ends, the necrotic region is clearly observable (Figure 6.9). The shape of the necrotic region is noticeably affected by the presence of blood vessels.

**Effect of Tumor Cell Quiescent pH**

$pH_{T}^{Q}$ is the pH below which tumor cells enter the quiescent state and stop dividing. Figure 6.11 shows the effect of this parameter on the temporal evolution of $R_{T}$. It is clear that when tumor cells are more tolerant of acidic environment (lower $pH_{T}^{Q}$), the tumor can grow more efficiently. A lower $pH_{T}^{Q}$ also means, however, that tumor cells will not reduce their rate of secreting lactic acid from $H_{p}^{T,A}$ to $H_{p}^{T,Q}$ until the local environment is more acid. Since the lethal pH for tumor cells is fixed at $pH_{T}^{D}$, there will
inevitably be more necrotic cells in the tumor interior. This is exactly what is shown by the plots in Figure 6.12.

**Effect of Host Vascularity**

Host vascularity $\phi_r$ is a quantitative indication of the host tissue’s ability of supplying glucose and removing lactic acid. In order to use the computer-generated capillary networks (CGCN) to study the effect of $\phi_r$ on tumor growth, we must first demonstrate that when all the model parameters are the same, simulations using the real capillary network shown in Figure 6.4 ($\phi_r = 1.43\%$) and the CGCN in Figure 6.6 ($\phi_r = 1.41\%$) can produce results that are acceptably close to each other. Figure 6.13 and Figure 6.14 show the difference in $R_T$, $N_T$, and $\varphi_{necrosis}$ for such a comparison. We can see that the $R_T$ curves share a common initial phase, but the CGCN curve deviates earlier and the difference in the final value of $R_T$, $R_{T,\text{final}}$, is $3.34\%$. Similar differences exist in $N_T$ and $\varphi_{necrosis}$ (Figure 6.14). There are two possible reasons for this discrepancy: (a) the topology of the two capillary networks is different, and (b) the values of their vascularity are not exactly the same. Nevertheless, we think the differences are small enough for us to use the CGCN’s to study the effect of $\phi_r$ on tumor growth.

Using the bifurcating distributive algorithm, we have generated two CGCN’s with $\phi_r = 8.06\%$ and $11.62\%$, respectively. Simulation runs similar to those in Figure 6.7 are carried out for these two capillary networks. Figure 6.15 shows that the transition tumor cell acid production rate $H_{P,\text{trans}}^{T,A}$ increases with increasing $\phi_r$. Thus, for host tissues with denser vasculature, the tumor needs to have a higher acid production rate in order to start the
aggressive growth. The combined effect of $H_p^{T,*}$ and $\phi_v$ on $R_{T, \text{final}}$ is shown in Figure 6.16. We can see that when $\phi_v$ is higher, the tumor can grow larger after $H_p^{T,*}$ increases above $H_{p, \text{trans}}$. This can be explained by Figure 6.17 which indicates that a lower portion of the tumor is necrotic when $\phi_v$ is higher. This means the tumor can have a higher $H_p^{T,*}$ without suffering too much necrosis in its interior. The general trend revealed by these results is consistent with the findings of the Patel et al. study. The values of $H_p^{T,*}$ in our study, however, are much closer to the experimental results.

**Effect of Tumor Dependence on Glucose Supply**

As mentioned earlier, the difference in normal and tumor cells’ dependence on nutrient supply can be reflected by their values of the saturation constants in the Monod kinetics (Eqn. (6.3)). In this study, we fix $K^N$ at 0.6022 mole/m3 and change $K^T$ from 0.01 mole/m3 to 10 mole/m3. The results of $R_{T, \text{final}}$ are plotted against $K^T$ in Figure 6.18. The value of $R_{T, \text{final}}$ asymptotically approaches a maximum as $K^T$ decreases, reflecting the characteristic of the Monod kinetics. Obviously, the tumor can grow more efficiently when it is less dependent on the supply of glucose. We should mention that such control of cell proliferation is not possible in the Patel et al. model because the state of cells in their cellular automaton does not include a division counter.
Figure 6.7: Tumor growth curves for various values of tumor cell acid production rate $H_p^{T,A}$ (shown in legend) when the real three-dimensional capillary network shown in Figure 6.4 is used. The other model parameters are the same as in Table 6.
Figure 6.8: The temporal evolution of the total number of tumor cells $N_T$ (Panel A) and the fraction $\phi_{\text{necrosis}}$ of necrosis in $\phi_{\text{necrosis}}$ (Panel B) for the simulation runs shown in Figure 6.7.
Figure 6.9: The temporal evolution of cell distribution in the tumor system for the case $H_{p}^{T,A} = 1.0 \times 10^{-4}$ mM/s in Figure 6.7. Color key: white – capillary cells, dark blue – active normal cells, light gray blue – necrotic normal cells, bright red – active tumor cells, dark brown – necrotic tumor cells.
Figure 6.10: The temporal evolution of the pH field in the tumor system for the case $H_p^{T,A} = 1.0 \times 10^{-4}$ mM/s in Figure 6.7.
Figure 6.11: Tumor growth curves for three different values of tumor cell quiescenting pH $\text{pH}_Q$ when the real three-dimensional capillary network shown in Figure 6.4 is used. The other model parameters are the same as in Table 6.
Figure 6.12: The temporal evolution of the total number of tumor cells $N_T$ (Panel A) and the fraction of necrosis $\varphi_{\text{necrosis}}$ (Panel B) for the simulation runs shown in Figure 6.11.
Figure 6.13: Tumor growth curves when the real three-dimensional capillary network shown in Figure 6.4 and when the computer-generated capillary network shown in Figure 6.6 are used. The former is labeled “RCN, $\phi_v = 1.43\%$” and the latter is labeled “CGCN, $\phi_v = 1.41\%$.” The other model parameters are the same as in Table 6.
Figure 6.14: The temporal evolution of the total number of tumor cells $N_T$ (Panel A) and the fraction of necrosis $\phi_{\text{necrosis}}$ (Panel B) for the simulation runs shown in Figure 6.13.
Figure 6.15: The transition tumor cell acid production rate $H_{P,\text{trans}}^{T,A}$ for the three different values of host vascularity $\phi_v$ of computer-generated capillary networks used in this study.
Figure 6.16: The combined effect of tumor cell acid production rate $H_p^{T,A}$ and host vascularity $\phi_v$ on the final value of tumor’s radius of gyration $R_{T,final}$. The other model parameters are the same as in Table 6.
Figure 6.17: The combined effect of tumor cell acid production rate $H_{p}^{T,A}$ and host vascularity $\phi_v$ on the final value of the fraction of tumor necrosis $\varphi_{\text{necrosis,final}}$. The other model parameters are the same as in Table 6.
Figure 6.18: The effect of tumor cells’ saturation constant in the Monod kinetics on the final value of tumor’s radius of gyration $R_{T,final}$. The other model parameters are the same as in Table 6.
6.5  Summary

The hybrid tissue growth model developed in the previous chapters is modified for the study of the acid-mediated early-stage tumor growth in this chapter. Our motivation to do so is to see if our model’s ability of describing complex, three-dimensional vasculature could overcome the limitations of existing two-dimensional models in this area. Because of the similarity between the tumor system and the perfused bioartificial tissues, only minor modifications are necessary for our model to be applied to this new practical problem. The CA model is able to include the multiple types of cells in the tumor system and one more diffusion-reaction PDE for lactic acid is added to compute the temporal evolution of the pH field. A real three-dimensional capillary network is generated from available data and is used for parametric studies on the effect of tumor cells’ acid production rate, quiescenting pH, and dependence on glucose supply. To investigate the effect of host vascularity on tumor growth, a bifurcating distributive algorithm is used to generate tree-like three-dimensional capillary networks with adjustable vascularity. Our simulations can predict tumor growth behavior similar to clinical observations. Moreover, our model is shown to be a significant improvement over the existing two-dimensional models in two aspects: (a) the tumor acid production rates needed in our model to maintain normal pH field in the tumor system and to facilitate tumor growth are much closer to reported experimental values; (b) our model can predict the universal existence of necrosis in tumors even though the time of its
appearance depends on the values of other parameters such as host tissue vascularity.
CHAPTER 7  CONCLUSIONS AND 
SUGGESTIONS FOR FUTURE 
WORK

7.1  Conclusions

This work presents a comprehensive hybrid computer model simulating the cell population and mass transfer dynamics during tissue growth processes. Based on a systematic analysis of the underlying mechanism for diffusion-controlled tissue growth, the model is built with three major components:

(1). A stochastic discrete model that simulates the migration and proliferation of individual cell, as well as cell-cell collisions.

(2). Transient and three-dimensional partial differential equations that describe the diffusion, convection, consumption and, possibly, secretion (e.g., lactic acid by tumor cells) of nutrients, growth factors, or other important substances in tissue systems.

(3). Equations that show how local concentrations of nutrients or growth factors modulate cell behavior.

The development of each model component is described in detail and realistic model parameters obtained from our own experiments or the literature are used to study two applications:
• The optimal design of perfusion channels used to mitigate the adverse effects of mass transport limitations
• The acid-mediated early-stage tumor growth.

These two seemingly very distinct problems have similar fundamental characteristics. In both systems, tissue growth is affected by the interplay between cell behavior and the concentration profiles of important nutrients and other substances.

The discrete component of our model captures the essential features of individual cell activities, allowing a detailed description of cell migration and proliferation. It is also capable of imitating cell interactions, such as cell-cell collisions and contact inhibition. Simulations that use only this model component indicate that cell motility and the initial seeding mode are important (yet often ignored) factors affecting the rate and pattern of tissue growth. With its ability to describe the behavior of individual cells, the discrete model can tell us how changes in cell-level properties will affect the dynamics of a cell population.

The diffusion-reaction PDE’s are established following the chemical engineering analysis of the transport and reaction processes during tissue growth. Various boundary conditions can be applied to reflect differences in bioreactor configuration and culture conditions. The PDE’s are discretized with an implicit-explicit scheme and the resulting linear systems are solved with a highly efficient parallel GMRES solver from PETSc. The correctness of this PDE-solving process is confirmed by comparing our numerical results to the analytical results for a three-dimensional diffusion-reaction
problem that can be solved with Finite Fourier Transforms. Concentrations computed by this method are then used to modulate the behavior of individual cells in the above discrete model. Cell proliferation is modulated according to a Monod-like kinetic expression. Due to the lack of experimental data, however, cell migration speed is modulated using a simple assumption.

The hybrid model is first used to study the growth of bioartificial tissues under conditions leading to nutrient depletion. Simulation results indicate that large tissue size, low nutrient diffusivity, high cell uptake rate and low nutrient concentration in the culture media lead to severe transport limitations and have serious adverse effects on both the growth rates and the structure of bioartificial tissues.

The incorporation of perfusion channels is one of the proposed methods for alleviating the adverse effects of diffusional limitations in order to promote tissue growth. However, the selection of optimal channel placement and size leads to an interesting optimization problem. In order to solve this problem for large tissues, the optimization problem is formulated and solved on a representative unit element of the entire tissue. Our results indicate the existence of an optimal channel diameter for each set of cell parameters and culture conditions. As diffusional limitations become more severe, larger perfusion channels are needed and the value of the achievable final cell volume fraction decreases.

The second practical problem we studied was the acid-mediated growth of solid tumors. Our major motivation for this effort is to see if our model, capable of describing the complex, three-dimensional vasculature in
tumor systems, is superior to existing two-dimensional models in answering the following two questions:

- Are the model predictions of tumor acid production rates needed to maintain normal pH levels and to facilitate tumor growth closer to experimental values than the existing two-dimensional models?
- Can our model predict the universal appearance of necrotic regions in the tumor interior?

First, a discrete representation of a real three-dimensional capillary network was generated from literature data. In order to study the effect of host vascularity on tissue growth, however, tree-like three-dimensional capillary networks with adjustable overall vascularity were also generated with a bifurcating distributive algorithm. Our simulations produced tumor growth curves that are similar to those observed clinically. The range of tumor cell acid production rate was closer to experimental values and necrotic regions appeared in all the simulation runs, even though the time of their appearance was affected by tumor cell acid production rate and host vascularity.

Our work shows the great potential and versatility of computer models in simulating diffusion-controlled tissue growth processes. The theoretical guidance provided by such models makes it possible for us to follow a rational approach either to solve design problems or to interpret clinical observations. Computer models like the one presented here may one day become indispensable tools for a wide variety of biotechnological and biomedical engineering applications.
7.2 Suggestions for Future Work

To improve our tissue growth model, future work should focus both on experimental studies aiming at a better understanding of the underlying physical and biological processes, and on theoretical efforts to develop and solve more realistic models of such processes.

Future experimental studies should quantify the effect of nutrient concentrations on the migration of mammalian cells. The time-lapse video microscopy procedure and digital image analysis previously developed in our lab\textsuperscript{[95]} can be used to monitor cell trajectories in culture media with various nutrient concentrations and to extract cell locomotory parameters (especially migration speed). It will be more desirable if such studies can be carried out in three-dimensional matrices made from natural materials such as collagen gel, because such matrices are more relevant to the growth of the three-dimensional tissues that we are modeling.

For the theoretical part of future work, I have recommendations for both the perfusion model and the tumor growth model. For the perfusion model, I recommend including oxygen as another vital substance that modulates tissue growth. Mammalian cells have high demand on oxygen and according to its availability, cells may alter their metabolic pathways of utilizing glucose and glutamine\textsuperscript{[207]}. The supply of oxygen has been found essential for the cultivation of engineered cartilage in bioreactors\textsuperscript{[208, 209]}. The published modeling studies in this area, however, are mostly for steady state and are thus not capable of describing the dynamic process of the tissue growing from the initial seeding to a steady state\textsuperscript{[210]}. Such a task requires a
hybrid model like ours. Adding oxygen to the model will not be very complicated. The basic diffusion-reaction PDE will be the same as the one for glucose. The Michaelis-Menten kinetics is also applicable for the consumption of oxygen by cells. It must be noted, however, that the consumption of glucose will need to be adjusted according to local oxygen concentration.

In addition to oxygen, one of more key growth factors (such as epithelia growth factor (EGF), platelet-derived growth factors (PDGF) and transforming growth factor-β (TGF-β)) should also be incorporated into the perfusion model. Such growth factors may affect the proliferation and/or migration of mammalian cells\textsuperscript{[211-216]}. Mathematical models describing the interaction of such growth factors with receptors on cell surface and the subsequent intracellular transduction of the mitogenic and/or chemotactic signals often consist of a set of coupled ordinary differential equations (ODE’s)\textsuperscript{[112, 217]}. These ODE’s will need to be solved on each cell during every PDE loop. Work on this topic has already started.

As to the modeling of tumor growth, I suggest that the process of angiogenesis be considered. The mechanism and function of tumor-induced angiogenesis is now well understood\textsuperscript{[41, 42, 218]}. As we have shown in Chapter 6, the vascular network plays an important role in regulating tumor growth. It will thus be a significant improvement if the process of angiogenesis can be incorporated to our model. Angiogenesis is induced by vascular endothelial growth factor (VEGF) secreted by tumor cells\textsuperscript{[219, 220]}. VEGF diffuses from the tumor site to the nearby native blood vessels and stimulates the sprouting of new blood vessels. This is obviously another
diffusion-reaction process and the concentration profile of VEGF can be computed in the same way the glucose concentration and pH field are computed. The sprouting of new blood vessels can be considered as a discrete process, with new endothelial cells growing towards the direction of higher VEGF concentration. Existing continuous models have been successful to a certain extent in simulating tumor-induced angiogenesis, but have difficulty in describing the complex vascular structures\textsuperscript{[221]}. From the work presented in Chapter 6, our hybrid approach might better suit this task and it is certainly worthwhile to explore the possibility of applying our model to this exciting and important area in the future.
APPENDIX-A

ALGORITHMS FOR INTER-PROCESS COMMUNICATION IN THE PARALLEL CELLULAR AUTOMATON MODEL

A.1 Parallel Processing

The parallelization of our CA model was originally implemented by Belgacem B. Youssef\textsuperscript{[222]} using the Message Passing Interface (MPI). Parallelism is introduced by dividing the work among multiple nodes at each time step. More specifically, the cellular grid is divided among the available processors by a slab decomposition along the $z$ dimension (Figure A-1). Let the number of processors be $P$. If $P$ divides $N_z$, all the subdomains will have equal size consisting of $N_x \times N_y \times \frac{N_z}{P}$ sites. If, however, $N_z = c_z P + r_z$, the cellular grid is divided into $r_z$ subdomains of size $N_x \times N_y \times \left(\frac{N_z}{P}\right) + 1$ and $P - r_z$ subdomains of size $N_x \times N_y \times \left\lfloor \frac{N_z}{P} \right\rfloor$. Each processor stays responsible for the local subdomain throughout the simulation with possible exchange of data with neighboring processors.

As mentioned in Chapter 2, each computational site in our CA model can hold at most one cell. We name this rule the “One-Site-One-cell” (1S1C) rule. This constraint does not cause any problem in the sequential code. The single node working on the problem owns the entire cellular grid
and, thus, knows the state of every site. Before allowing a cell to move or divide, it can scan its neighborhood to determine whether the specified action is allowed. In the parallel algorithm using the above slab decomposition scheme, however, the processing of occupied sites at ownership borders becomes difficult because of possible violations of the \textit{ISIC} rule. Figure A-2 shows that each process has to know whether or not a site in the boundaries shared with neighbors is occupied or not in order to correctly choose the next direction/position when a cell in the boundaries moves/divides. Thus, for each subdomain we create two "ghost" layers, one at the top and the other at the bottom (Figure A-2). The bottom ghost layer of process $i$ ($1 \leq i \leq P$) records boundary layer $n_z$ of the subdomain in \textit{mypred} (the process mapped before \textit{myself}, the current process) and the top ghost layer of process $i+1$ records boundary layer 1 of the subdomain in \textit{mysucc} (the process mapped after \textit{myself}). The information in the ghost layers will be used, if necessary, for the movement/division of a cell.

A change of cell position in a ghost/boundary layer can happen at any time during the movement/division routine. Such changes can be caused by cell movement/division in a boundary layer (e.g., when a cell in layer $n_z$ moves south) or in the layer next to it (e.g., when a cell at layer $n_{z-1}$ moves up). Such information must be sent to and be processed by the corresponding process to avoid violating the "\textit{ISIC}" rule (i.e., 2 cells moving to 1 site) like the one shown in Figure A-2: a cell occupying site $k$ of process $P_{i+1}$ at time $t'$ wants to move into the empty site $k+1$ at the next time level $t'^{+1}$. At the same time step, the cell occupying site $j$ of process $P_i$ may decide to move into the site $\overline{k+1}$ which is the ghost image of site $k+1$. If
this conflict between the two competing cells is not handled appropriately, the parallel algorithm will place two cells into the same site and, as a result, one cell will be lost.

For the possible violation of the ISIC rule described above, Youssef developed an algorithm which includes a feedback mechanism that (a) randomly selects one of the two competing cells to take the target site, and (b) re-calculates and re-executes the movement/division for the rejected cell[222]. While very efficient, Youssef’s algorithm (called Algorithm 1 hereafter) cannot completely prevent the cell loss caused by violations of the ISIC rule. Next, we will analyze the reason for this problem and describe the two algorithms (Algorithms 2 and 3) that we have developed as an attempt to prevent cell loss while maintaining the efficiency of the parallel program. Since the algorithms of the movement and the division routines are very similar, we will use the movement routine as an example for the following description and analysis.
Figure A-1: The slab decomposition of a cellular grid with dimensions on $P$ processors. The dashed lines indicate shared boundaries between two neighboring processors.
Figure A-2: The relation between boundary layers and ghost layers in the slab decomposition scheme and an typical example of violation of the ISIC rule due to the ownership problem on shared boundaries.
A.2 Algorithms for Inter-Process Communication

A.2.1 Algorithm 1

In this algorithm, if a cell is moving within the local subdomain, the movement is executed immediately. For cells crossing over to a neighboring subdomain, an additional step is taken to record the starting position and the direction of their movement. After all the local cells have been considered, a message carrying the information about the recorded boundary-crossing movement is sent to the corresponding neighbor. Each process then checks the destination site for every recorded movement in the message. If a local cell has moved into that site, a random number is generated to decide which cell, the local one or the one from a neighboring subdomain, can take that site. The rejected cell will need to have its movement re-calculated and re-executed. This inter-process communication scheme imposes very little system overhead, but it does not completely prevent violation of the ISIC rule. Cell loss may happen when a rejected cell finds no empty site in its original neighborhood. Such incidents, however, are very rare and their impact is kept to a very level. The basic steps of Algorithm 1 are as follows:

\[
\text{WHILE the specified volume coverage has not been reached} \\
\quad \text{WHILE not all cells in the local subdomain have been considered} \\
\qquad \text{Select the next cell in the subdomain according to a random order.} \\
\quad \text{Calculate cell movement.} \\
\qquad \text{IF the cell is not crossing over to a neighboring subdomain THEN} \\
\qquad \quad \text{Execute its movement.}
\]
ELSE
    Record the cell’s current position and the calculated new cell state in a matrix $M_{crossing\_to\_mypred}$ for cells attempting to cross over to $mypred$ and a matrix $M_{crossing\_to\_mysucc}$ for cells attempting to cross over to $mysucc$.
ENDIF
Send $M_{crossing\_to\_mypred}$ to $mypred$ and $M_{crossing\_to\_mysucc}$ to $mysucc$.
Receive $M_{crossing\_from\_mypred}$ from $mypred$ and $M_{crossing\_from\_mysucc}$ from $mysucc$.
FOR each cell recorded in $M_{crossing\_from\_mypred}$ and $M_{crossing\_from\_mysucc}$
    Decide whether a cell that has crossed over from a neighbor will be accepted (into an empty site of the subdomain) or rejected due to violation of the $ISIC$ rule with a local cell.
    Record the position of a rejected cell in a matrix $M_{rejected\_to\_mypred}$ for cells going back to $mypred$ and in a matrix $M_{rejected\_to\_mysucc}$ for cells going back to $mysucc$.
ENDFOR
Send $M_{rejected\_to\_mypred}$ to $mypred$ and $M_{rejected\_to\_mysucc}$ to $mysucc$.
Receive $M_{rejected\_by\_mypred}$ from $mypred$ and $M_{rejected\_by\_mysucc}$ from $mysucc$.
FOR each cell recorded in $M_{rejected\_by\_mypred}$ and $M_{rejected\_by\_mysucc}$
    Recalculate cell movement from the cell’s original position.
    Execute cell movement.
ENDFOR
ENDWHILE
Send layer 1 to $mypred$ and lay $n_z$ to $mysucc$.
Receive layer 0 from $mypred$ and layer $n_z+1$ from $mysucc$.
Update the time step.
ENDWHILE
After a parallel algorithm like Algorithm 1 is developed, we usually evaluate it by checking its correctness and performance. The correctness is reflected by the difference between the parallel results and the sequential results obtained with the same set of model parameters. Among all the simulation results, we use the data of cell volume fractions for the correctness check. Let $\kappa_3(r)$ and $\kappa_{p,i}(r)$ be the cell volume fraction values obtained at the $r$-th iteration by the sequential algorithm and a parallel algorithm $i$, respectively. Since we will have a total of three parallel algorithms, $i$ can be 1, 2, or 3. We will consider the following vectors of cell volume fraction computed by the sequential and the parallel algorithms:

Sequential: \[ \overline{K}_S = [\kappa_3(1), \kappa_3(2), \ldots, \kappa_3(m_S)] \]

Parallel: \[ \overline{K}_{p,i} = [\kappa_{p,i}(1), \kappa_{p,i}(2), \ldots, \kappa_{p,i}(m_P)] \], where $i = 1, 2, 3$

where $m_S$ and $m_P$ are the number of iterations needed by the sequential and the parallel programs to reach confluence, respectively. The relative error of the parallel algorithm is then defined as follows:

\[ e_i = \frac{\| \overline{K}_S - \overline{K}_{p,i} \|_2}{\| \overline{K}_S \|_2} \], where $i = 1, 2, 3$

where $\| x \|_2$ is the $L_2$ norm of a vector $x = [x_1, x_2, \ldots, x_m]$ defined as: \[ \| x \|_2 = \sqrt{x_1^2 + x_2^2 + \ldots + x_m^2} \]. When the two vectors $\overline{K}_S$ and $\overline{K}_{p,i}$ do not have the same number of components (i.e. the two algorithms reach confluence after different numbers of iterations), the longer vector is truncated before
computing the relative errors according to the above equation. The performance of a parallel algorithm is usually reflected by two values: Speedup and Efficiency. If the CPU time needed by the sequential algorithm and the parallel algorithm using $P$ processors to run a simulation is $T_0$ and $T_P$, respectively, we will have

$$\text{Speedup} = \frac{T_0}{T_P} \text{ and Efficiency} = \frac{T_0}{P \times T_P}.$$  

For a typical simulation run, Algorithm 1 gives results with small relative errors (Figure A-5A) and has good performance (Figure A-5B). However, a simple "Movement-Only" experiment reveals that there is a hidden problem. In a $100 \times 100 \times 100$ cellular grid, $5 \times 10^5$ cells are uniformly seeded ($\kappa_0 = 0.5$) and the model parameters are set so that cells can move, but cannot divide. The simulation is then executed on 4 processors. Since cells cannot divide, we should see them moving around while their total number remains constant, no matter how long the simulation runs. Figure A-3 indicates, however, that there is cell loss as the simulation continues. Although the percentage of the lost cells is relatively small (about 1% after 10 days), it reveals a correctness problem that must be investigated. Going back to the detail of Algorithm 1, we find that if a cell, which is returning to its original site because of a rejection, finds that both that site and all the neighboring sites are now occupied by other local cells, it will have no place to go and will thus be lost. Thus, the way Algorithm 1 handles the boundary ownership conflicts may fail under certain circumstances. Such
circumstances, however, cannot be kept from arising in the parallel program if we are to maintain the CA model's stochasticity by randomizing the order at which cells are considered in every subdomain. How, then, can cell loss be prevented?

The cell loss in Algorithm 1 is caused by two reasons: (a) the messages about the cell movement that may affect a boundary or ghost layer are not exchanged immediately after the movement happens. Such communication is delayed until all the local cells have been considered; (b) local cells are allowed to move during the delay just mentioned. These two facts, when combined together, make it possible for one boundary cell's original site to be taken by another local cell while it tries to cross over to a neighboring process but is then rejected. If either of these two reasons can be prevented, there should be no cell loss. The time delay between the sending and the receiving of a message, however, is inevitable in the distributed-memory system that we are using. Figure A-4 shows that a sent message has to line up in a queue in the system buffer and wait for its turn to be received by the target process. No matter how short the waiting time in the system buffer is, it cannot be reduced to zero. Thus, we have to focus on the second reason in order to prevent cell loss in the parallel algorithm. This realization leads to the development of Algorithm 2.
Figure A-3: The temporal evolution of cell volume fraction from a “Movement-Only” experiment indicates that there is cell loss in Algorithm 1. This simulation starts with $5 \times 10^5$ cells being seeded uniformly in a $100 \times 100 \times 100$ cellular grid (50% seeding) and runs on 4 processors. Cells can move as usually, but cannot divide.
Figure A-4: The delay between the moment a message is sent in one process and the moment the same message can be retrieved by another process is inevitable in a distributed-memory system.
A.2.2 Algorithm 2

In Algorithm 2, each process communicates with a neighbor and pauses the execution of local cell movement whenever it considers a boundary site where the movement of a cell may affect a position in the shared boundaries. More specifically, a process sends a message to the neighbor that may be affected when considering a boundary site, and then waits for a message from the same process using the blocking MPI_RECV. This guarantees that if a boundary cell is trying to move, no local cells will be able to move (possibly into that boundary cell’s original site) until that movement is confirmed by the corresponding neighbor. Such cell movement may originate from a boundary layer or the layer next to it. Thus, we make every site in layer 1, 2, \(n_z\)-1 and \(n_z\) execute the sending and receiving operations to ensure that every process knows exactly how many messages \((2 \times N_x \times N_y)\) are coming from each neighbor so that there will not be deadlock in the inter-process communication. For each process, there is then the problem of receiving and executing a message first or calculating and executing local cell movement first. We have to give certain processes (e.g., the even-numbered) the priority by allowing their cell movement to be executed first. For example, the main operations of an even numbered process \(P_{2i}\) during the \(k\)-th simulation time step can be performed in the following order when it considers a site in layer 1, 2, \(n_z\)-1 or \(n_z\):

(1). Calculate the movement for the local cell occupying that site

(2). Execute the calculated movement

(3). Send a message about the movement
(4). Receive a message
(5). Update the subdomain according to the received message.

If that site is not occupied, steps 1 and 2 should be skipped. The odd-numbered processes $P_{2i+1}$ execute these operations in a different order:
(1). Receive a message
(2). Update the subdomain according to the received message
(3). Calculate cell movement
(4). Execute cell movement, and
(5). Send a message

If that site is not occupied, steps 3 and 4 should be skipped. If the above orders are followed throughout the entire simulation, the even-numbered processes will have an accumulated priority. To even things out, the orders are switched between the even-numbered and the odd-numbered processes at every other iteration. Next, we describe the detailed actions of an even-numbered process $P_{2i}$ in Algorithm 2 during the $k$-th simulation time step:

\begin{verbatim}
WHILE the specified volume coverage has not been reached
    WHILE not all cells in the local subdomain have been considered
        Select the next site in the subdomain according to a random order.
        IF the site is in layers 1, 2, $nz$-1 or $nz$ of the subdomain, THEN
            IF the site is occupied by a cell, THEN
                Calculate cell movement.
                Execute cell movement.
            ELSE
                If the site is not occupied, THEN
                    Calculate cell movement.
                    Execute cell movement.
        ELSE
            Calculate cell movement.
            Execute cell movement.
    END
END
\end{verbatim}
ENDIF
Send a message to the corresponding neighbor.
Receive a message from the same neighbor.
Update subdomain according to the received message.
ELSE
IF the site is occupied by a cell, THEN
  Calculate cell movement.
  Execute cell movement.
ENDIF
ENDIF
Count the number of cells in the subdomain.
Calculate volume coverage of the entire tissue.
Update the time step of the simulation.
ENDWHILE
Update the time step.
ENDWHILE

The “Movement-Only” experiment indicates that there is no cell loss in Algorithm 2. The simulations results are also closer to the sequential results than Algorithm 1 (Figure A-5A). The performance of this algorithm, however, is very low (Figure A-5B). In fact, it takes much longer time to run the parallel program than the sequential program because the values of Speedup in Figure A-5B are much lower than 1.0. The reason for such low performance is the blocking MPI_RECV following the sending of each cell movement message. Because local cells are considered in a randomized order (see the pseudo-code above), the MPI_RECV may cause one process to pause at a site in layer 1, 2, nz-1 or nz after sending a message while the neighboring process from which a message is expected is working on non-
boundary sites. It may take a long time before that neighboring process considers a site in layer 1, 2, \(n_z-1\) or \(n_z\) of its local subdomain and sends the expected message.

To make Algorithm 2 practically useful, we must improve its efficiency. The key here is to eliminate the blocking MPI_RECV while still preventing any violation of the \textit{ISIC} rule. This is what we will try to do in Algorithm 3.

\section*{A.2.3 Algorithm 3}

In Algorithm 3, each process communicates with a neighbor whenever it considers a cell (not a site as in Algorithm 2) who is moving and whose movement may affect a position in the shared boundaries. We should emphasize again that such cells may exist in a boundary layer or the layer next to it. The messages are received with the non-blocking MPI_Irecv (instead of the blocking MPI_RECV in Algorithm 2) to allow the overlapping of computation and communication. When a cell in layer 1, 2, \(n_z-1\) or \(n_z\) is considered, a message is sent to the corresponding neighbor, but the process does not pause until it receives a message from that neighbor. Instead, calculation and execution of movement continue for local cells and MPI_Irecv is performed frequently to check for incoming messages. Because of the overlapping of computation and communication, however, it is still possible for the \textit{ISIC} rule to be violated. Thus, a feedback mechanism similar to the one used in Algorithm 1 is developed to prevent such incidents
As described before, the ownership conflict is only possible when one local cell tries to move into a boundary site (either from another site in the boundary layer or from a site in the layer next to the boundary layer) and another cell tries to move into the same site from a neighboring process. When such conflicts arise, Algorithm 1 generates a random number to decide which cell can take it. In this algorithm, however, we always give one of these two cells the priority. This makes it possible to develop a feedback mechanism that can completely prevent actual violation of the ISIC rule. We divide the cell movement that may cause an ownership conflict into two types. The first type includes those only affecting the local subdomain (e.g., a cell in layer \( n_z \)-1 moves up or a cell in layer \( n_z \) moves south). We give such cell movement the priority if it conflicts with the movement of a cell attempting to cross over from a neighboring process. This means, for example, the cell at site \( k \) in Figure A-2 will have the priority over site \( j \). After being calculated, the cell’s movement is executed immediately and, even if the boundary layer is not affected, a “notification” message is sent to the corresponding neighbor. This is to eliminate the possibility of deadlock in inter-process communication. The other type of cell movement includes those affecting a neighboring subdomain (e.g., a cell in layer \( n_z \) moves up or a cell in layer 1 moves down). Such cell movement can only be executed if it does not conflict with any cell in the target neighboring process. Otherwise, the cell has to return to its original site and try to move to a neighboring site in the local subdomain. To make sure that a boundary-crossing cell will not fall in the aforementioned situation of having no place to go, its calculated movement is not executed immediately.
Instead, its intention is recorded in an "inquiry" message which is then sent to the corresponding neighbor. That neighbor, upon receiving the "inquiry" message, will check the destination site in its local subdomain and send back an "accepted" feedback message (if the destination is empty) or a "rejected" feedback message (if the destination is occupied). A cell can execute its calculated movement after being "accepted", but will have to re-calculate and re-execute its movement if it is "rejected".

For the above inter-process communication to be successful, it is necessary that every process knows the numbers of expected "inquiry" and "notification" messages as well as the numbers of expected feedback messages from both mypred and mysucc. According to the description above, the number of moving cells in layers n_z−1 and n_z of mypred is the total number (N_{mympred.top}) of "inquiry" and "notification" messages that will come from mypred. Similarly, the number of moving cells in layers 1 and 2 of mysucc is the total number (N_{mysucc.btm}) of "inquiry" and "notification" messages that will come from mysucc. The numbers of feedback messages (N_{mympred.feedback} from mypred and N_{mysucc.feedback} from mysucc), however, cannot be known beforehand because we do not know how many local cells will attempt to cross over to a neighboring subdomain. Thus, we must use N_{mympred.feedback} and N_{mysucc.feedback}, numbers that are initially set to zero, to record the unanswered "inquiry" messages by mypred and mysucc, respectively. Therefore, N_{mympred.feedback} is incremented or decremented by one when myself sends an "inquiry" message to or receives a feedback message from mypred. Similarly, N_{mympred.feedback} is incremented or decremented by one
when \textit{myself} sends an “inquiry” message to or receives a feedback message from \textit{mysucc}.

The detail of Algorithm 3 is described in the following pseudo-code:

\begin{itemize}
\item \textbf{WHILE} the required volume coverage has not been reached
\item \hspace{1cm} Scan local subdomain in a random order.
\item \hspace{1cm} Count the number of cells $N_{\text{myself, btm}}$ in layers 1 and 2 that may move or divide.
\item \hspace{1cm} Count the number of cells $N_{\text{myself, top}}$ in layers $n_z-1$ and $n_z$ that may move or divide.
\item \hspace{1cm} Send $N_{\text{myself, btm}}$ to \textit{mypred} and $N_{\text{myself, top}}$ to \textit{mysucc}.
\item \hspace{1cm} Receive $N_{\text{mysucc, btm}}$ from \textit{mysucc} and $N_{\text{mypred, top}}$ from \textit{mypred}.
\item \hspace{1cm} Set $N_{\text{mypred, feedback}}$ and $N_{\text{mysucc, feedback}}$ to zero.
\end{itemize}

\begin{itemize}
\item \textbf{WHILE} not all sites in the subdomain have been considered or not all the expected messages have been received
\item \hspace{1cm} \textbf{IF} not all sites in the subdomain have been considered \textbf{THEN}
\item \hspace{2cm} Select the next site according to a random order.
\item \hspace{2cm} \textbf{IF} the site is occupied \textbf{THEN}
\item \hspace{3cm} \textbf{IF} the cell is not in layer 1, 2, $n_z-1$ or $n_z$ of the subdomain \textbf{THEN}
\item \hspace{4cm} Calculate cell movement.
\item \hspace{4cm} Execute cell movement.
\item \hspace{3cm} \textbf{ELSE}
\item \hspace{4cm} Calculate cell movement.
\item \hspace{4cm} \textbf{IF} the cell does not attempt to cross over to a neighboring subdomain \textbf{THEN}
\item \hspace{5cm} Execute cell movement.
\item \hspace{5cm} Send a “notification” message to the corresponding neighbor for it to update the affected ghost layer.
\end{itemize}
ELSE
    Send an “inquiry” message to the corresponding neighbor.
    Increase by one the number \( N_{mypred,feedback} \) or \( N_{mysucc,feedback} \)
    of expected feedback messages from that neighbor.
ENDIF
ENDIF
ENDIF
ENDIF

IF not all expected messages have been received THEN
    Perform an MPI_I_RECV.
    Check the returned flag of MPI_I_RECV to see if there is an incoming message.
    IF there is no incoming message THEN
        Cancel the receive operation with MPI_CANCEL.
    ELSE
        Receive the message.
        Check the type of the message.
        IF the received message is an “inquiry” message THEN
            IF the destination site is occupied THEN
                Send a “rejected” feedback message to the corresponding neighbor.
            ELSE
                Update the boundary layer according to the message.
            ENDIF
            Send an “accepted” feedback message to the corresponding neighbor.
            Reduce by one the number \( N_{mypred,top} \) or the number \( N_{mysucc,btn} \) of expected messages from that neighbor.
    ENDIF
ENDIF
ELSEIF the received message is a “notification” message
THEN
    Update the ghost layer according to the received message.
    Reduce by one the number $N_{mypred,top}$ or the number $N_{mysucc,btm}$
    of expected messages from the corresponding neighbor.
ELSE (i.e., the received message is a “feedback” message)
    Reduce by one the number $N_{mypred,feedback}$ or the number
    $N_{mysucc,feedback}$ of expected feedback messages from the
    corresponding neighbor.
    IF it is an “accepted” message THEN
        Execute cell movement.
    ELSE (i.e., it is a “rejected” message)
        Recalculate cell movement for the rejected cell.
        Execute the calculated cell movement.
        Send a “notification” message to the corresponding
        neighbor.
    ENDIF
ENDIF
ENDIF
ENDIF
ENDIF
ENDWHILE
    Update the time step.
ENDWHILE

The “Movement-Only” experiment confirms that there is no cell loss
in Algorithm 3. Figure A-5 indicates that generally Algorithm 3 is only
slightly less accurate than Algorithm 2 and much more accurate than
Algorithm 1. Moreover, its performance is much better than Algorithm 2
and is comparable to Algorithm 1. Results from a more systematic
comparison of algorithms 1 and 3 are shown in Figure A-6, Figure A-7, and Figure A-8. We can see from Figure A-6 that the accuracy of both algorithms improves as the size of the cellular grid increases. This is because the fraction of boundary layers in the entire cellular grid decreases for larger grids if the same number of processors are used. In light of the same argument, the relative error of Algorithm 1 increases when more processors are used for a fixed cellular grid. It is interesting to see, however, that the relative error of Algorithm 3 appears to be relatively unaffected by the number of processors. Figure A-7 and Figure A-8 indicate that, as expected, Algorithm 1 always has better performance than Algorithm 3.

A.3 Conclusions

The algorithm for inter-process communication is very important for the parallel implementation of our CA model because it determines both the correctness and the performance of the parallel program. We have successfully found the solution to a hidden problem in Youssef’s algorithm that may lead to the violation of the ISIC rule. It should be emphasized, however, that the accuracy of Youssef’s algorithm is well acceptable and its good performance can make it a better choice when not many processors are available for simulations on large cellular grids.
Figure A-5: Comparing the accuracy and performance of the three parallel algorithms for a 120×120×120 cellular grid. Other run parameters: Seeding density =1%; cell migration speed = 50 μm/hr; persistence time = 2 hr; average division time = 12 hr.
Figure A-6: The effect of the size of the cellular grid and the number of processors on the accuracy of the results from parallel algorithms 1 and 3. The other model parameters are the same as in Figure A-5.
Figure A-7: The speedup exhibited by parallel algorithms 1 and 3 for two grid sizes. The other model parameters are the same as in Figure A-5.
Figure A-8: The efficiency exhibited by parallel algorithms 1 and 3 for two grid sizes. The other model parameters are the same as in Figure A-5.
APPENDIX-B  ANALYTICAL SOLUTION FOR THE DIFFUSION-REACTION PROBLEM

We want to find the analytical solution for the following PDE:

\[
\frac{\partial \theta}{\partial \tau} = \left[ \frac{\partial^2 \theta}{\partial x^2} + \frac{\partial^2 \theta}{\partial y^2} + \frac{\partial^2 \theta}{\partial z^2} \right] - k_1 \cdot \theta - k_2 \quad (B.1)
\]

B.C.: \( \theta = 0 \) on the six surfaces

I.C.: \( \theta = 1 \) for \( \bar{x} \in (0, 1), \bar{y} \in (0, 1), \bar{z} \in (0, 1), \tau = 0 \)

Rearrange Eqn. (B.1) as

\[
L_x[\theta] = \frac{\partial^2 \theta}{\partial x^2} = \frac{\partial \theta}{\partial \tau} - \frac{\partial^2 \theta}{\partial y^2} - \frac{\partial^2 \theta}{\partial z^2} + k_1 \cdot \theta + k_2 \quad (B.2)
\]

The operator \( L_x[\ ] \) and the associated homogeneous boundary conditions define the eigenvalue problem

\[
L_x[\phi] = \frac{\partial^2 \phi}{\partial x^2} = -\lambda \phi, \quad \bar{x} \in (0,1)
\]

\( \phi(0) = 0, \phi(1) = 0 \)

which gives the eigenvalues
\[ \lambda_n = n^2 \pi^2, \quad n = 1, 2, \ldots \]

and the orthonormal eigenfunctions:

\[ \varphi_n(x) = \sqrt{2} \sin(n \pi \bar{x}), \quad n = 1, 2, \ldots \]

*A subset \{v_i, \ldots, v_j\} of a vector space \(V\), with the inner product \(<,>\) is called orthonormal if \(<v_i, v_j> = 0\) when \(i \neq j\). That is, the vectors are mutually perpendicular. Moreover, they are all required to have length one: \(<v_i, v_i> = 1\). Here the inner product is \(<v_i, v_j> = \int_0^1 v_i \cdot v_j d\bar{x}\).*

Taking the FFT of Eqn. (B.2) gives

\[ \int L_x[\theta] \varphi_n(\bar{x}) d\bar{x} = \int \left[ \frac{\partial \theta}{\partial \tau} - \frac{\partial^2 \theta}{\partial y^2} - \frac{\partial^2 \theta}{\partial z^2} + k_1 \cdot \theta + k_2 \right] \varphi_n(\bar{x}) d\bar{x} \quad (B.3) \]

We can simplify the lhs and rhs of this equation:

\[
\text{lhs} = \int \theta(\bar{x}, \bar{y}, \bar{z}, \tau) L_x[\varphi_n] d\bar{x} \\
= \int \theta(\bar{x}, \bar{y}, \bar{z}, \tau) \left[ -\lambda_n \varphi_n(\bar{x}) \right] d\bar{x} \\
= -\lambda_n \theta_n(\bar{y}, \bar{z}, \tau)
\]

where \(\theta_n(\bar{y}, \bar{z}, \tau) = F_x[\theta(\bar{x}, \bar{y}, \bar{z}, \tau)] = \int \theta(\bar{x}, \bar{y}, \bar{z}, \tau) \varphi_n(\bar{x}) d\bar{x}\) is the FFT of \(\theta(\bar{x}, \bar{y}, \bar{z}, \tau)\) with respect to the variable \(\bar{x}\). The rhs of Eqn. (6.4.4) takes the form
\[ \text{rhs} = \frac{\partial \theta_n}{\partial \tau} - \frac{\partial^2 \theta_n}{\partial y^2} - \frac{\partial^2 \theta_n}{\partial z^2} + k_1 \cdot \theta_n + \Omega_n \]

where \[ \Omega_n = \frac{\sqrt{2}}{n \pi} \left[ 1 - (-1)^n \right] \cdot k_2 \]

So we have

\[ -\lambda_n \theta_n = \frac{\partial \theta_n}{\partial \tau} - \frac{\partial^2 \theta_n}{\partial y^2} - \frac{\partial^2 \theta_n}{\partial z^2} + k_1 \cdot \theta_n + \Omega_n \]

which may be rearranged to give

\[ L_y[\theta_n] = \frac{\partial^2 \theta_n}{\partial y^2} = \frac{\partial \theta_n}{\partial \tau} - \frac{\partial^2 \theta_n}{\partial z^2} + (\lambda_n + k_1) \cdot \theta_n + \Omega_n \quad \text{(B.4)} \]

The eigenvalue problem corresponding to \( L_y[\ ] \) and its associated homogeneous boundary conditions are

\[ L_y[\psi] = \frac{\partial^2 \psi}{\partial y^2} = -\mu \psi, \quad y \in (0, 1) \]

\[ \psi(0) = 0, \psi(1) = 0 \]

The eigenvalues and orthonormal eigenfunctions are
\[ \mu_m = m^2 \pi^2, \quad m = 1, 2, \ldots \]
\[ \psi_m(y) = \sqrt{2} \sin(m\pi y) \quad m = 1, 2, \ldots \]

Taking the FFT of Eqn. (B.4) leads to

\[ \int_0^1 L_y[\theta_n(y)] \psi_m(y) \, d\bar{y} = \int_0^1 \left[ \frac{\partial \theta_n}{\partial \tau} - \frac{\partial^2 \theta_n}{\partial \bar{z}^2} + (\lambda_n + k_1) \cdot \theta_n + \Omega_n \right] \psi_m(y) \, d\bar{y} \quad (B.5) \]

As we have done before, both sides of Eqn. (B.5) can be simplified:

\[ \text{lhs} = \int_0^1 L_y[\theta_n(y)] \psi_m(y) \, d\bar{y} \]
\[ = \int_0^1 \theta_n(\bar{y}, \bar{z}, \tau) L_y[\psi_m(y)] \, d\bar{y} \]
\[ = \int_0^1 \theta_n(\bar{y}, \bar{z}, \tau) \left[ -\mu_m \psi_m(y) \right] \, d\bar{y} \]
\[ = -\mu_m \theta_{nm}(\bar{z}, \tau) \]

where \[ \theta_{nm}(\bar{z}, \tau) = F_y[\theta_n(\bar{y}, \bar{z}, \tau)] = \int_0^1 \theta_n(\bar{y}, \bar{z}, \tau) \psi_m(y) \, d\bar{y} \] is the FFT of \[ \theta_n(\bar{y}, \bar{z}, \tau) \] with respect to the variable \( \bar{y} \). The rhs of Eqn. (B.5) takes the form

\[ \text{rhs} = \frac{\partial \theta_{nm}}{\partial \tau} - \frac{\partial^2 \theta_{nm}}{\partial \bar{z}^2} + (\lambda_n + k_1) \cdot \theta_{nm} + \Omega_{nm} \]

where \[ \Omega_{nm} = \frac{\sqrt{2}}{n\pi} \frac{\sqrt{2}}{m\pi} \left[ 1 - (-1)^n \right] \cdot \left[ 1 - (-1)^m \right] \cdot k_2. \]

Thus we have
\[-\mu_m \theta_{nm} = \frac{\partial \theta_{nm}}{\partial \tau} - \frac{\partial^2 \theta_{nm}}{\partial z^2} + (\lambda_n + k_1) \cdot \theta_{nm} + \Omega_{nm}\]

which may be rearranged to give

\[
L_z[\theta_{nm}] = \frac{\partial^2 \theta_{nm}}{\partial z^2} = \frac{\partial \theta_{nm}}{\partial \tau} + (\lambda_n + \mu_m + k_1) \cdot \theta_{nm} + \Omega_{nm} \tag{B.6}
\]

Repeating the procedure followed above, now for the \(z\) variable, gives rise to the eigenvalue problem:

\[
L_z[\xi] = \frac{\partial^2 \xi}{\partial z^2} = -\eta \xi, \quad z \in (0,1)
\]

\[\xi(0) = 0, \quad \xi(1) = 0\]

which has eigenvalues and orthonormal eigenfunctions

\[
\eta_p = p^2 \pi^2, \quad p = 1, 2, \ldots
\]

\[
\xi_p(z) = \sqrt{2} \sin(p \pi z), \quad p = 1, 2, \ldots
\]

Taking the FFT of Eqn. (B.6) gives, as before, we obtain

\[-\eta_p \theta_{nmp} = \frac{d \theta_{nmp}}{d \tau} + (\lambda_n + \mu_m + k_1) \cdot \theta_{nmp} + \Omega_{nmp}\]
where
\[
\Omega_{nmp} = \frac{\sqrt{2}}{n\pi} \frac{\sqrt{2}}{m\pi} \frac{\sqrt{2}}{p\pi} \left[1 - (-1)^n \right] \left[1 - (-1)^m \right] \left[1 - (-1)^p \right] \cdot k_2
\]
\[= 8 \frac{\sqrt{2}}{n\pi} \frac{\sqrt{2}}{m\pi} \frac{\sqrt{2}}{p\pi} \cdot k_2, \quad n, m \text{ and } p = 1, 2, ...
\]

If we let \( \Theta_{nmp} = \lambda_n + \mu_m + \eta_p + k_1 \), the above equation can be rearranged to give
\[
\frac{d\Theta_{nmp}}{d\tau} = -\Theta_{nmp} \cdot \Theta_{nmp} + \Omega_{nmp}
\]  
(B.7)

where \( \Theta_{nmp}(\tau) = F \left[ \Theta_{nm}(\bar{z}, \tau) \right] = \int_0^1 \Theta_{nm}(\bar{z}, \tau) \bar{\xi}_p(\bar{z}) d\bar{z} \) is the FFT of \( \Theta_{nm}(\bar{z}, \tau) \) with respect to the variable \( \bar{z} \). Note that \( \Theta_{nmp} \) is the triple FFT of the original variable \( \Theta(\bar{x}, \bar{y}, \bar{z}, \tau) \), obtained by taking three successive transforms of \( \Theta \) with respect to the variable \( \bar{x}, \bar{y}, \) and \( \bar{z} \), respectively. The solution of Eqn. (B.7) is
\[
\Theta_{nmp}(\tau) = \left[ \Theta_{nmp}(0) - \frac{\Omega_{nmp}}{\Theta_{nmp}} \right] \cdot \exp\left[ -\Theta_{nmp}(\tau) \right] + \frac{\Omega_{nmp}}{\Theta_{nmp}}
\]  
(B.8)

The only unknown in expression (B.8) is \( \Theta_{nmp}(0) \), which we determine now.

For this, recall the initial condition for \( \Theta \):

\[
\Theta = 1 \text{ for } \bar{x} \in (0, 1), \quad \bar{y} \in (0, 1), \quad \bar{z} \in (0, 1), \quad \tau = 0
\]
So

\[ \theta_{n}(y, z, 0) = \int_{0}^{1} \theta(x, y, z, 0) \phi_{n}(x) dx = \int_{0}^{1} 1 \cdot \phi_{n}(x) dx = \sqrt{2} \int_{0}^{1} \sin(n\pi x) dx = \frac{\sqrt{2}}{n\pi} \left[ 1 - (-1)^{n} \right] \]

Similarly,

\[ \theta_{nm}(z, 0) = \int_{0}^{1} \theta_{n}(y, z, 0) \psi_{m}(y) dy = \int_{0}^{1} \frac{\sqrt{2}}{n\pi} \left[ 1 - (-1)^{n} \right] \psi_{m}(y) dy = \frac{\sqrt{2}}{n\pi} \left[ 1 - (-1)^{n} \right] \sqrt{2} \int_{0}^{1} \sin(m\pi y) dy = \frac{\sqrt{2}}{n\pi} \frac{\sqrt{2}}{m\pi} \left[ 1 - (-1)^{n} \right] \left[ 1 - (-1)^{m} \right] \left[ 1 - (-1)^{p} \right] \]

and finally,

\[ \theta_{nmp}(0) = \int_{0}^{1} \theta_{nm}(z, 0) \eta_{p}(z) dz = \frac{\sqrt{2}}{n\pi} \frac{\sqrt{2}}{m\pi} \frac{\sqrt{2}}{p\pi} \left[ 1 - (-1)^{n} \right] \left[ 1 - (-1)^{m} \right] \left[ 1 - (-1)^{p} \right] = \frac{\Omega_{nmp}}{k_{2}} \]

By now, \( \theta_{nmp}(\tau) \) in Eqn. (B.8) is fully known:
\[ \theta_{nmp}(\tau) = \left[ \frac{1}{k_2} - \frac{1}{\Theta_{nmp}} \right] \cdot \Omega_{nmp} \cdot \exp\left[-\Theta_{nmp}\tau\right] + \frac{\Omega_{nmp}}{\Theta_{nmp}} \]

We now need to take the inverse transforms to obtain the solution for \( \theta(x, y, z, \tau) \). These are taken in the reverse order of variables. Thus

\[ \theta_{nm}(z, \tau) = F_{z}^{-1}\left[\theta_{nmp}(\tau)\right] = \sum_{p=1}^{\infty} \theta_{nmp}(\tau) \xi_p(z) \]

\[ \theta_n(y, z, \tau) = F_{y}^{-1}\left[\theta_{nm}(z, \tau)\right] = \sum_{m=1}^{\infty} \theta_{nm}(z, \tau) \psi_m(y) \]

and

\[ \theta(x, y, z, \tau) = F_{x}^{-1}\left[\theta_n(y, z, \tau)\right] = \sum_{n=1}^{\infty} \theta_n(y, z, \tau) \phi_n(x) \]

In explicit form:

\[
\theta(x, y, z, \tau) = \sum_{n=1,2}^{\infty} \sum_{m=1,2}^{\infty} \sum_{p=1,2}^{\infty} \left\{ \frac{64}{\pi^3 nmp} \sin(n \pi x) \sin(m \pi y) \sin(p \pi z) \cdot \left[ 1 - \frac{k_2}{\Theta_{nmp}} \right] \exp\left(-\Theta_{nmp}\tau\right) + \frac{k_2}{\Theta_{nmp}} \right\}
\]

The notation \( \sum_{j=1,2}^{\infty} \) means summation from 1 to \( \infty \) in steps of 2; that is, terms involving \( j = 1, 3, 5, \ldots \) are summed.
REFERENCES


