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**Tissue Engineering**  

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Tissue Engineering

A Major Qualifying Project Report
Submitted to The Faculty
of
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by

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Approved:

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Professor Sarah Olson

This report represents the work of WPI undergraduate students submitted to the faculty as evidence of completion of a degree requirement. WPI routinely publishes these reports on its website without editorial or peer review. For more information about the project program at WPI, please see http://www.wpi.edu/academics/understudies/project-learning.html
Abstract

In this project, we develop study the behavior of a collagen network around fixed cells in a two-dimensional model. A mathematical distribution is used to determine a random direction $\theta$ which the collagen molecules can use to move via a random walk. We show the versatility of using both discrete and continuous mathematics to create a working collagen model. We create a discrete model which is used to locate and plot cells, as well as determine if conditions are appropriate for collagen to form and move. To study the variability of different distributions, we plot the number of collagen able to be produced, as well as keep track of the occurrences in which overcrowding takes place. To study the interaction between cell movement and collagen production, we plot collagen and evaluate which areas of the domain become overcrowded. Results are discussed in connection with the diffusion equation in order to extrapolate further adaptations to existing code.
Executive Summary

Biology is the study of living organisms, and the cell is the basic structural, functional, and biological unit of all known living organisms. In biology, tissue is an aggregate of cells in an organism that have a similar structure and function. Engineering is the branch of science and technology concerned with design, building, and creation of structures. Tissue Engineering combines the biological knowledge of the building blocks of the human body, cells, and the science behind structural engineering in order to construct functional tissue.

Cells secrete the building blocks of the protein collagen. Collagen bind together to form into an Extracellular Matrix. In doing this the collagen provide a sturdy support system for the cell. By studying the behavior of cells, the conditions necessary to produce collagen, and the interaction between the two by using two-dimensional discrete Random Walk Models, more can be learned about the Extracellular Matrix.

The goal of this project is to create a discrete Random Walk Model of collagen and cellular production and movement. One of the most important sources of collective motion on the molecular level is diffusion, the result of random motion of individual molecules. Random Walk Models attempt to mathematically recreate or simulate motion. Motion as defined in mathematics, is either discrete or continuous. Discrete motion occurs in steps, and allows for abrupt changes in direction, where continuous motion has to be fluid and uninterrupted. Though in mathematics, it is simple enough to describe continuous motion in terms of discrete mathematics. We will show how the random motion of our discrete model can be discussed in terms of continuous mathematics by formulating a Partial Differential Equation.

When simulating the movement of cells within a Random Walk Model, the Uniform Distribution calculates $\theta$ which is used to direct the movement of the cellular model. In many models the Uniform Distribution is used to facilitate random movement. This study uses various distributions such as the von Mises Distribution as well as the Wrapped Normal Distribution to calculate the desired $\theta$ used in helping direct the motion of the cells and collagen. This is done in order to compare the two distributions to the most commonly used Uniform Distribution and evaluate if they provide any type of statistically significant variance when calculating $\theta$. Two different variations of the model are simulated in order to determine whether further restrictions on the movement of collagen will increase or decrease the occurrence of overcrowding.
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Chapter 1

Introduction

1.1 Cells and Collagen

Cell movement or motility is a highly dynamic phenomenon that is essential to a variety of biological processes such as the development of an organism, wound healing, cancer metastasis, and immune response [1]. Cell movement is primarily driven by the actin network beneath the cell membrane, and can be divided into three general components: protrusion of the leading edge of the cell, adhesion of the leading edge and de-adhesion or detachment occurring at the cell body and rear, and cytoskeletal contraction to pull the cell forward [1]. In this study, cellular movement will be determined by randomly generating a direction of motion, and evaluating whether the given cell or collagen protein can move a predetermined step length.

Within the cell, two types of peptide chains, known as preprocollagen, are formed and release a triple helical structure known as procollagen. This is then secreted by the cell, where it undergoes a transformation and becomes tropocollagen molecules. These tropocollagen molecules group together to form collagen fibrils, and multiple collagen fibrils group together to form collagen fibers [8]. The collagen created will be one of three types, the most common is type I collagen, which have enormous tensile strength; that is, such collagen can be stretched without being broken [8]. In this study cells secrete the basic building blocks of the fibrous protein type I collagen, which moves in a random walk about each cell and interacts with the cells and other collagen proteins.

The knowledge of life sciences and engineering that allow us to develop biological and synthetic substitutes which can be used to maintain or repair damaged tissue in the human body, is the definition of tissue engineering [7]. The regeneration of tissues for clinical use has already, to a limited extent, been implemented. Tissue regeneration is essentially related to complex bio processes of tissue growth that involves cell, cell population, and tissue level events. Cell-cell and cell-environment interactions such as adhesion, migration, proliferation, and diffusion, all determine cell growth rates and influence the final tissue formation [3]. Random Walk Models (RWM) and Collagen Models are used to better understand and replicate movement on the cellular level.

The goal of this project is to create a discrete RWM of collagen and cellular production and movement. One of the most important sources of collective motion on the molecular level is diffusion, the result of random motion of individual molecules. We will show how the random motion of our discrete model can be discussed in terms of continuous mathematics by formulating a Partial Differential Equation (PDE). First, we must better understand the connection between random motion and diffusion, to
do this we must take into account conservation of energy and create a balance equation. The simplest way to think about this is by evaluating the movement of cells in a one dimensional area.

1.2 Random Walk Models

A Random Walk is a mathematical object, known as a stochastic or random process, which describes a path that consists of random steps on some mathematical plane. For example, the path traced by a molecule as it moves through a liquid or gas, the search path taken by an animal foraging in nature, or the movement of cells through tissue can all be approximated by using RWMs even though they may not be entirely random in reality. RWMs began simply as one dimensional timelines as seen in figure (2.2), and have evolved into the two and three dimensions. Some of the first two-dimensional (2D) models attempted to simulate cell population dynamics by using non motile cells which could proliferate. This was a fine starting point but neglected to take into consideration the adverse effects of contact inhibition between cells [2]. Cheng and associates attempted to create a three-dimensional model (3D) and set guidelines to determine the random movement of cells. They randomly generated a migration index $m$ which would generate a random integer between 1 and 7 with predetermined directions of north, south, east, and west [2] where as in our model we generate the direction of movement via an angle $\theta$ from $[0, 2\pi]$ around the cell in 2D. We also investigate how the results vary when different distributions are used to find the angle $\theta$. Our model also asses the surrounding environment to ensure that the cell or collagen has the appropriate space needed to move in the given direction.

RWMs can be quite complex; in this study we create a very simplistic model and focus more on the production, movement, and interaction of collagen with stationary cells. The goal of this project was to create a discrete RWM of collagen and cellular production and movement. One of the most important sources of collective motion on the molecular level is diffusion, the result of random motion of individual molecules. We show how random motion as shown in our discrete model can be discussed in terms of continuous mathematics by formulating a Partial Differential Equation (PDE).
Chapter 2

Methods

2.1 One-dimensional Balance Equation

Figure 2.1: Balance equations are obtained to calculate concentration of particles $c(x, t)$ along a tube, much like the flow of cells throughout tissue. (a) Assuming the tube has a uniform cross-sectional area $A$, (2.3) results from the balancing $\sigma(x, t)$ of the given section (b) of length $\Delta x$.

First, consider the open interval $(x, x + \Delta x)$, letting $x$ represent some arbitrary location along a tube. The concentration of particles at any given location $x$ at time $t$, denoted by $c(x, t)$. The number of particles which travel into and out of the open interval, known as the flux, denoted by $J(x, t)$. $J$ is denoted in vector notation, in 1D it is a quantity which has a direction to the right, pointing toward the cross sectional area at $x$ and out toward the right at $x + \Delta x$. The degradation or creation of particles already contained within the interval, denoted by $\sigma(x, t)$.

Denote the number of particles entering the cross section at by $J(x, t)$, and the particles leaving the cross section by $J(x + \Delta x, t)$. Consider the cross-sectional area $A$ as shown in figure (2.1), calculate the volume of the open interval, of length $\Delta x$, which will be equivalent to $A\Delta x$. Using the given variables, we can extrapolate the equation,

$$\frac{\partial}{\partial t} [c(x, t)A\Delta x] = J(x, t)A - J(x + \Delta x, t)A \pm \sigma(x, t)A\Delta x.$$  \hspace{1cm} (2.1)
In the aforementioned balance equation $c$ depends on two variables $x$ and $t$, or location and correlates to the rate of change of concentration at a particular location $x$. By taking the derivative of equation (2.1) with respect to time, this is known as a partial derivative. Notice that the signs of the flux in equation (2.1) correspond with the net change in particles over the interval $[x, x + \Delta x]$. By assuming the volume remains constant, and dividing through by $A\Delta x$, we get

$$\frac{\partial c(x, t)}{\partial x} = \frac{J(x, t) - J(x + \Delta x, t)}{\Delta x} \pm \sigma(x, t).$$

(2.2)

Taking the limit as $\delta x$ approaches zero reduces the width of the cross-section to an arbitrarily small value, yielding the end result, which is the one-dimensional balance equation

$$\frac{\partial c(x, t)}{\partial x} = -\frac{\partial J(x, t)}{\partial x} \pm \sigma(x, t).$$

(2.3)

This basic form of the balance law will apply to coming equations.

### 2.2 Random Motion and Diffusion Equation

Ficks first law relates the diffusive flux to the concentration under the assumption of equilibrium. It also hypothesizes that flux moves from regions of high concentration to regions of low concentration, with a magnitude proportional to the concentration gradient. Ficks first law is:

$$J = -D\nabla c.$$  

(2.4)

Recalling that $J$ is the diffusion flux. The diffusion coefficient represented by $D$, with dimensions expressed as area per unit of time or meters squared per second ($m^2/s$). The diffusion coefficient is the net migration due to diffusion down the gradient, which is a vector of partials, away from the most concentrated locations. In the 1D case diffusion flux is given by $J = -D \left[ \frac{\partial c}{\partial x} \right]$, substituting equation (2.4) into equation (2.3) results in

$$\frac{\partial c(x, t)}{\partial t} = \frac{\partial}{\partial x} \left[ D \frac{\partial c(x, t)}{\partial x} \right].$$

(2.5)

Consider equation (2.5) in 3D, then

$$\frac{\partial c}{\partial t} = \nabla \cdot (D\nabla c),$$

(2.6)

$$= \nabla \cdot D \left[ \frac{\partial c}{\partial x}, \frac{\partial c}{\partial y}, \frac{\partial c}{\partial z} \right],$$

(2.7)

$$= \left[ \frac{\partial}{\partial x}, \frac{\partial}{\partial y}, \frac{\partial}{\partial z} \right] \cdot D \left[ \frac{\partial c}{\partial x}, \frac{\partial c}{\partial y}, \frac{\partial c}{\partial z} \right].$$

(2.8)

If the previous equation is evaluated under the assumption that the diffusion coefficient, $D$, is constant then it can be moved outside of the dot product:

$$= D \left[ \frac{\partial^2 c}{\partial x^2}, \frac{\partial^2 c}{\partial y^2}, \frac{\partial^2 c}{\partial z^2} \right],$$

(2.9)

$$= D \left[ \frac{\partial^2 c}{\partial x^2}, \frac{\partial^2 c}{\partial y^2}, \frac{\partial^2 c}{\partial z^2} \right],$$

(2.10)

$$\frac{\partial c}{\partial t} = D\nabla^2 c.$$  

(2.11)
2.2.1 Random Walk and Diffusion Equation

Visualize a line segment as shown in figure (2.2) with a concentration, \( c(x, t) \), of cells at an arbitrary location \( x \), where cells move one step to the left, \( x - \Delta x \), or to the right, \( x + \Delta x \). The average step length being \( \Delta x \) for every time interval \( k \). Let the probability of moving left be, \( \lambda_l \), or to the right, \( \lambda_r \), be equal; that is, \( \lambda_l = \lambda_r = \frac{1}{2} \). This can be written in the form of a discrete equation which describes the change in the number of particles located at the arbitrary location \( x \).

![Figure 2.2: A cluster of particles located at \( x \), randomly step a predetermined length of \( \Delta x \), augmented by the probability of shifting left or right, denoted by \( \lambda_l, \lambda_r \).](image)

Let \( C(x_0, t_0 + \Delta t) \Delta x \) be the number of particles located within the interval \([x, x + \Delta x]\) at time \( k \), then

\[
C(x, t + k) = C(x, t) + \lambda_r C(x - \Delta x, t) - \lambda_r C(x, t) + \lambda_l C(x + \Delta x, t) - \lambda_l C(x, t).
\]

(2.12)

The previous equation (2.12), Calculates the concentration of particles located at \( x \), at time \( t \), iteration \( k \) by taking into consideration the concentration at time \( t = 0 \), and accounting for the probability of particles moving one step length \( \Delta x \) to the right, from their location at \( x - \Delta x \) at time \( t \). Subtracting the probability that particles move one step length \( \Delta x \) from their location at the origin. Accounting for the probability that particles move one step length \( \Delta x \) to the left from their postilion at \( x + \Delta x \) and subtracting the Probability that particles take a step length \( \Delta x \) to the left from the origin.

The discrete equation (2.14) can be written as a Taylor-series expansion, this will allow the discrete equation to be re-written as a PDE, and therefore expressing diffusion as a continuous mathematical equation. The Taylor-series can be used here because the point being evaluated is centered at the origin. Taylor-series is only effective when approximating values in close proximity to the origin, it is not effective when making broad extrapolations. Therefore the Taylor-series equation which can be used is as follows:

\[
C(x, t + k) = C(x, t) + \frac{\partial C}{\partial t} k + \frac{1}{2} \frac{\partial^2 C}{\partial t^2} k^2 + \cdots,
\]

(2.13)

\[
C(x \pm \Delta x, \tau) = C(x, t) \pm \frac{\partial C}{\partial t} \Delta x \pm \frac{1}{2} \frac{\partial^2 C}{\partial x^2} \Delta x^2 \pm \cdots.
\]

(2.14)

Then by substituting both equations (2.15) and (2.16) into the discrete equation (2.14) and using the probabilities of \( \lambda_l = \lambda_r = \frac{1}{2} \), the result is the PDE

\[
\frac{\partial c}{\partial t} k + \frac{1}{2} \frac{\partial^2 c}{\partial t^2} k^2 + \cdots + \frac{1}{2} \frac{\partial^2 c}{\partial x^2} \Delta x^2 + \frac{1}{4} \frac{\partial^4 c}{\partial t^4} \Delta x^4 + \cdots.
\]

(2.15)
By dividing through by \( k \), and taking the limit as \( k \to 0 \), and the limit as \( \Delta x \to 0 \), such that

\[
\frac{(\Delta x)^2}{2k} = \text{constant} = D, \tag{2.16}
\]

Then the result is

\[
\frac{\partial c}{\partial t} = \frac{(\Delta x)^2}{2k} \frac{\partial^2 c}{\partial x^2} - D \frac{\partial^2 c}{\partial x^2}. \tag{2.17}
\]

This can be further simplified to

\[
\frac{\partial c}{\partial t} = 2D \frac{\partial^2 c}{\partial x^2} - D \frac{\partial^2 c}{\partial x^2}. \tag{2.18}
\]

Further simplification results in the equation

\[
\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2}. \tag{2.19}
\]

This equation is equal to equation (2.7), therefore showing the versatility of transitioning between discrete and continuous mathematics. The same conclusion can be reached multiple ways, and communicate the same ideas using different mathematical methods. Extensions of the RWM for numerous other special cases are described in [9].

### 2.3 CollagenECMModels

Recall that collagen is a protein, specifically a protein made up of amino-acids, which are in turn built of carbon, oxygen, and hydrogen. Collagen, however, is just one protein that comprises or is contained within the Extracellular Matrix (ECM). In biology, the ECM is a collection of extracellular molecules secreted by cells that provide structural and biochemical support to the surrounding cells, which provides mechanical support and physical separation of tissues, it also regulates key biological processes [3]. By effectively modeling collagen production, more can be learned about the growth and distribution of the ECM. In this model the ECM will be comprised solely of collagen molecules, to provide support to the existing modeled cells.

Dallon and associates propose two ways to mathematically model fibroblast-collagen interaction [5], both models are force based and model the cells as individual entities with discrete attachment sites; however, the collagen lattice is more interconnected and formed by triangulating nodes to form a fibrous structure (a), as shown in figure (2.4). In the collagen fiber model, the nodes are not triangulated, are less interconnected, and the collagen fibers are modeled as a string of nodes (b), as shown in figure (2.4).

Interconnection refers to the state or strength of connection, or to the potential to connect two nodes. Nodes, as shown by the red stars in figure (2.4) represent the vertices of connection between two collagen springs. The figure above demonstrates the natural phenomenon of triangulation in (a), which occurs when three individual components, in this case collagen springs, link together at a common vertex or node [6].Triangulation creates a stronger ECM, compared to the collagen strings model which lacks any form of triangulation. Within discrete RWM’s it is possible to simulate complex interconnected triangulated collagen ECM’s. However, in this model we will take a simpler approach, and will not take into consideration the interconnection of collagen.
Figure 2.3: (a) depicts a model cell interacting with a collagen lattice, where (b) depicts a model cell interacting with collagen strings. The lattice and strings are shown by the grey lines, where the cell membrane is represented by the black lines. The red stars represent nodes. In (a) the nodes are located at the intersections of the grey lines or collagen lattice. In (b) the nodes are not restricted to intersections, as the grey lines, representing the collagen strings do not form vertices. (a) shows the triangulation of the interconnected collagen lattice represented by the blue lines which intersect to form triangular structures, while (b) shows that collagen strings lack any form of triangulation, as the blue lines are just that, lines.

2.4 Distributions

When simulating the movement of cells within a RWM, the Uniform Distribution is the most commonly used distribution to calculate $\theta$ which is used to direct the movement of the cellular model. In this model, $\theta$ is calculated by calling a random number using the Uniform Distribution, which is then multiplied by $2\pi$ in order to create an angle $\theta$ which ranges from $[0, 2\pi]$. During this study, we experiment by using different distributions to calculate $\theta$, in order to see if they will create any variably differences in the model. Specifically, we are going to take a look at two Circular Distributions: The first being the von Mises distribution and the second being the Wrapped Normal. Probability Distributions allow us to work and transition between discrete and continuous mathematics much like PDE’s and Taylor Series as discussed in earlier sections.

A Probability Density Function (PDF), as shown in figure 2.4(a) below, is a probability law for a continuous random variable, let us call this variable $X$ (in discrete terms we would use a probability mass function). The probability law defines the chances of the random variable taking a particular value $X$ such that $P(X = x)$. This definition however is not valid for continuous random variables as the probability at any given point is zero. Therefore, we could use an alternative form such that PDF $= P(x - t < X \leq x)$ as $t$ tends to zero.

A Cumulative Distribution Function (CDF), as shown in figure 2.4(b), is simply the probability up to a particular value of the random variable $X$. We generally denote this using $F$, such that $F = P(X \leq x)$ far any value of $x$ in the $X$ space. Unlike a PDF it is defined for both discrete and continuous random variables.
Figure 2.4: (a) Is the graphical representation of the Probability Density Function of a Uniform Distribution. The red distribution curve represents a standard normal uniform distribution curve with a mean $\mu = 0$ and variance $\sigma^2 = 1$. The green distribution curve represents a negatively skewed standard normal uniform distribution curve which shifts to the left with a mean $\mu = -2$ and slightly smaller variance $\sigma^2 = 0.5$ than normal. The blue distribution curve represents a standard normal uniform distribution curve skewed by positive Kurtosis, which means that it mean $\mu = 0$ has a higher probability of being chosen, and the variance $\sigma^2 = 0.2$ is considerably smaller than normal. Lastly the black distribution curve represents a standard normal uniform distribution curve skewed negatively by Kurtosis, which means that it mean $\mu = 0$ has a lower probability of being chosen, and the variance $\sigma^2 = 5.0$ is considerably larger than normal. (b) Is the graphical representation of the Cumulative Distribution Function of a Uniform Distribution. The red, green, blue, and black distribution functions have the same mean and variance $(\mu, \sigma^2)$ as shown in (a), (b) is simply a secondary way to graphically represent the same discrete data in a continuous form.

2.5 Circular Distribution ($\theta$)

In probability and statistics, a Circular distribution or polar distribution is a probability distribution of a random variable whose values are angles, usually taken to be in the range $[0, 2\pi)$. Circular distributions can be used to draw random angles for either the direction of movement or turning angle at each step of 2D RW [4]. Commonly used circular distributions are described in the coming sections.

2.5.1 von Mises Distribution

In probability theory and directional statistics, the von Mises distribution (also known as the circular normal distribution or Tikhonov distribution) is a continuous probability distribution on the circle, and is commonly denoted by

$$f(x) = \frac{e^{\kappa \cos(x - \mu)}}{2\pi I_0(\kappa)} \quad (2.20)$$

for all $\kappa$ and for $-2\pi < \mu, 2\pi$. Where $I_0$ denotes the modified Bessel function of the first kind and
order 0. The graphical representation is similar to that of the standard normal uniform distribution as shown in figure 2.4.

### 2.5.2 Wrapped Normal Distribution

In probability theory and directional statistics, a Wrapped normal distribution is a wrapped probability distribution that results from the “wrapping” of the normal distribution around the unit circle infinitely many times. The wrapped normal distribution is defined by the PDF

\[
f(x; \mu, \sigma) = \frac{1}{\sqrt{2\pi\sigma}} \sum_{k=1}^{\infty} \exp \left( -\frac{(x + 2\pi k - \mu)^2}{2\sigma^2} \right),
\]

with \( x \) contained in \([0, 2\pi]\) with mean \( \mu \) contained in \([0, 2\pi]\), and the variance \( \sigma > 0 \). The graphical depiction is similar to that shown in figure 2.4.

The wrapped normal distribution is the standard normal distribution “wrapped” around a unit circle, and hence is easy to interpret and simulate. The von Mises distribution is easier to deal with analytically, however, and is similar to the wrapped normal distribution with the same mean cosine, so is often used instead of the wrapped normal. In three-dimensional space, a second angular direction \( \phi \) must also be specified, and spherical distributions are used. Spherical distributions and data are difficult to deal with (which may be the reason why many movement studies are restricted to two dimensions), although it is possible to use a vector-based approach [4].
Chapter 3

Results and Discussion

Figure 3.1 shows the temporal evolution of a cellular array which simulates cells producing of collagen in a 2D model. This simulation starts by randomly placing 500 cells within a 50 x 50 domain. The locations of the cells are generated randomly, and are represented by the little blue circles in figure 3.1(a), and are fixed throughout the duration of the simulation as recorded in figure 3.3(a). Each cell produces a randomly generated number of collagen at each time step, represented by the little red stars in figure 3.1(b-f), if conditions are appropriate. All collagen then generate a random direction of movement $\theta$ and evaluate if conditions are appropriate. If conditions are appropriate then the collagen move a prefixed step in the calculated direction $\theta$, if the conditions are not appropriate then the collagen remain in their current location. There are two conditions in which collagen are not allowed to move, the first is if the movement will cause the collagen to step outside of the boundaries of the array, and the second is if the collagen are attempting to move into the radius of the cell. As the iterations progress, it can be seen in figure 3.1(b-e) that more and more collagen movement is being restricted by the boundary. Once the number of collagen which cannot move has exceeded 25% of the total collagen contained within the domain, we define this as overcrowding.

The first simulations were run using the Random Normal Distribution, to randomly determine the direction $\theta$ of movement as previously discussed in chapter 2. The angles $\theta$ from $[0, 2\pi]$ were recorded and the number of occurrences of each angle $\theta$ from $[0, 2\pi]$ are shown in figure 3.2(a). The same was done for both the von Mises, and Wrapped Normal Distributions as shown in figure 3.2 (b) and (c) respectively. The mean $\mu$ and variance $\sigma^2$ were calculated for each distribution used based on data collected from 25 simulations. As the Random Normal Distribution is the most commonly used distribution in RWM’s, it was used to compare to the other two distributions. Hypothesis testing is used to determine statistically whether two sets of values have anything in common. I conducted a two tailed p-test to test whether any of the distributions are similar, therefore, under the assumption that the von Mises and Wrapped Normal Distributions would statistically vary from the Random Normal Distribution, we found the $p-value$ to be greater than 0.1 in both cases. Therefore, it can be said that using any one of the distributions will not provide any statistically significant different results. All distributions as seen in figure 3.2 were generated in an excel spread sheet using data stored in vectors and exported from matlab.

Figure 3.4 shows the temporal evolution of a cellular array which simulates cells production of collagen in a 2D model. This simulation starts by randomly placing 500 cells within a 50 x 50 domain. The locations of the cells are chosen similarly to the previous model, and are denoted by the small blue circles in Figure 3.4 (a). Each cell then produces a randomly generated number of collagen, given that the conditions are appropriate, unlike the previous model the collagen stay within a fixed distance from
the cell which can be seen more clearly in figure 3.4(c) and (d). The collagen then move around via randomly generated angles $\theta$ which are restricted as the collagen must remain within the fixed distance of the cell. Therefore, the circle of collagen as seen in figure 3.4 (d) can be compared to the 1D line segment in figure (2.2), and movement then is similar to the movement of particles as described in figure (2.2).

The first simulations were run for this variation of the 2D model, also used the Random Normal Distribution, to randomly determine the direction $\theta$ of movement. The angles $\theta$ from $[0, 2\pi]$ were recorded and the number of occurrences of each angle $\theta$ from $[0, 2\pi]$ are shown in figure 3.2(a). The same was done for both the von Mises, and Wrapped Normal Distributions as shown in figure 3.2 (b) and (c) respectively. The mean $\mu$ and variance $\sigma^2$ were calculated for each distribution used based on data collected from 25 simulations. There was a slight difference compared to the first mathematical model, but not a very large one. The Random Normal Distribution is the most commonly used distribution in RW’s, it was used to the statistically evaluate the other two distributions. In conducting a two-tailed $p$-test in order to determine if there was indeed some substantial variance between the distributions, we found that under the assumption that the von Mises and Wrapped Normal Distributions would statistically vary from the Random Normal Distribution, we found the $p$-value to be greater than 0.1 in both cases and therefore we can reject the null hypothesis that there is any significant variance between the distributions evaluated. Therefore it can be said that using any one of the distributions in either of the 2D model variations will not provide any statistically significant different results. All distributions as seen in figure 3.5 were generated in an excel spread sheet using data stored in vectors and exported from matlab.

Though the variance of the distributions does not denote any statistical significance, the rate of collagen production is noticeably different between the to variations of the model. The difference is clearly shown when comparing figure 3.6(b) and figure 3.3(b). In the first variation of the model, the collagen RW has little to no constraints which it must follow, where as in the second variation the collagen must remain within a prefixed distance of the cell. Therefore, accounting for the lower collagen production in the second variation of the model, and the increasing occurrence of overcrowding.
Figure 3.1: Initial locations of cells (a) and temporal evolution of a 50 x 50 domain simulating cells production of collagen in a 2D model. Snapshots (b-e) correspond to time steps $t = 25$, $t = 50$, $t = 75$, and $t = 100$ respectively. Snapshot (c) shows the first signs of potential overcrowding along the upper right hand corner of the domain, while (d) and (e) display the steady increase in collagen production. Snapshot (f) provides a look at an enlarged section of the domain to show that collagen do not in fact enter the radius of the cell, and allows shows the scattered and random movement of the collagen throughout the array.
Figure 3.2: The Random Normal Distribution (a) produced on average $\mu = 46$ of each angle $\theta$ from $[0, 2\pi]$ with a variance of $\sigma^2 = 7$. The von Misses Distribution (b) produced on average $\mu = 49$ of each angle $\theta$ from $[0, 2\pi]$ with a variance of $\sigma^2 = 7$. The Wrapped Normal Distribution produced on average $\mu = 51$ of each angle $\theta$ from $[0, 2\pi]$ with a variance of $\sigma^2 = 7$. Variance in (b) accounts for the highest percentage of angles $\theta$ calculated, though it contains the most obvious outlier, in only producing the angle $\theta = 3.52$ on average 6 times.
Figure 3.3: Cells are initialized once, and remain fixed throughout the duration of the simulation (a). Each cell produces a randomly generated number of collagen from 1 to 3, therefore steadily increasing the total number of collagen within the domain (b), this growth can be described linearly. During each time step a random direction $\theta$ must be generated for each collagen produced, then a random direction $\theta$ must be generated for each collagen present in the array (c), thus this vector of values can be expressed exponentially.
Figure 3.4: Initial locations of cells (a) and temporal evolution of a 50 x 50 domain simulating cells production of collagen in a 2D model. The production of collagen within a fixed distance 0.1 steps from the collagen (b). Snapshot (c) shows a 5 x 5 enlarged portion of the domain, depicting the ring of collagen formed around each cell. While snapshot (d) is a further enlarged portion of the domain, which shows that the individual collagen move separately at a prefixed distance of 0.1 away from the cell center. This is to simulate the movement of collagen which form attachments around the cell and move as one.
Figure 3.5: The Random Normal Distribution (a) produced on average $\mu = 44$ of each angle $\theta$ from $[0, 2\pi]$ with a variance of $\sigma^2 = 7$. The von Misses Distribution (b) produced on average $\mu = 48$ of each angle $\theta$ from $[0, 2\pi]$ with a variance of $\sigma^2 = 8$. The Wrapped Normal Distribution produced on average $\mu = 50$ of each angle $\theta$ from $[0, 2\pi]$ with a variance of $\sigma^2 = 7$. Variance in (b) accounts for the highest percentage of angles $\theta$ calculated, with no remarkable outliers.
Figure 3.6: Cells are initialized once, and remain fixed throughout the duration of the simulation (a). Each cell produces a randomly generated number of collagen from 1 to 3, therefore steadily increasing the total number of collagen within the domain (b), this growth can be described linearly. During each time step a random direction \( \theta \) must be generated for each collagen produced, then a random direction \( \theta \) must be generated for each collagen present in the array (c), thus this vector of values can be expressed exponentially. In order to plot this exponential relationship an empty vector had to ca
Chapter 4

Analysis

We have developed a 2D simulation model for describing cells production of collagen that moves via a RW model. Individual collagen move freely about the domain, two collagen may occupy the same space, but are restricted by the boundaries of the array, and are not allowed to travel within the radius of fixed cells. Three different mathematical distributions were used in order to generate the directions $\theta$ of the RW collagen. In comparing the various distributions, there was no evidence of statistically significant variance when calculating the directions $\theta$ of the RW collagen. Simulations show that in the absence of collagen in the immediate perimeter of the cell will lead to a greater production of collagen, as the cell has no indication on the number of collagen which it has already produced. This will lead to a surplus of collagen collecting around the boundary, taking a far longer time to overcrowd the entirety of the domain.

We also developed a variation to the previous 2D model in which the RW collagen produced by the cells more closely simulated a collagen lattice model. Collagen were restricted to a fixed distance from the radius of the cell from which it was produced, and therefore leaving less available space in the immediate proximity of the cell for new collagen. This also reduced the directions $\theta$ which could be assigned to certain collagen, therefore causing a more frequent occurrence of overcrowding. When run using the various distributions to calculate the random directions $\theta$ of the RW collagen, there was again no statistically significant difference between the variance of directions $\theta$ calculated. Simulations show that collagen which tend to stay closer together, allow the cell to terminate production sooner, and therefore the overcrowding is the result of a strongly formed collagen lattice producing a stable structure around the cell. In conclusion, RWM which use Random Normal, von Mises, or Wrapped Normal Distributions will not likely produce statistically significant variations when determining the direction $\theta$ of a particles RW.

Further adaptations which could be applied to the model include the ability of cells to proliferate, account for the collision and/or binding effect of collagen to one another or cells, or even perhaps to formulate a similar model using a mathematically modeled matrix to grant a more solid structure to each of the cells and collagen walking about the model.
Appendix A

MATLAB Code

CollagenProductionUpdate

```matlab
function [LocCollagenNew, CollagenNew, Angles, AnglesNew]=CollagenProductionUpdate(Loc,
CellsTotal,n,~,~,~,~,ciprolif)
CellsTotal(n);
CollagenNew=0;
Angles=[ ];
AnglesNew=0;
for i=1:CellsTotal(n)
    %generate random number of new collagen produced near cell i
    CollagenProduced=randi(2);
    NumberofNewCollagen=CollagenProduced(1);
    NumberofNewThetaCalculated=NumberofNewCollagen;
    for j=CollagenNew+1:CollagenNew+NumberofNewCollagen
        r=ciprolif;
        % Compute the random step directions.
        %going to consider using preallocating for speed
        Theta=(2*pi)*rand(1,1);
        Angles=[Angles; Theta];
        LocCollagenNew(j,1) = Loc(i,1)+r*cos(Theta);
        LocCollagenNew(j,2) = Loc(i,2)+r*sin(Theta);
    end
    CollagenNew=CollagenNew+NumberofNewCollagen;
    AnglesNew=AnglesNew+NumberofNewThetaCalculated;
end
```

CollagenLocationUpdate

```matlab
function [LocCollagen, Angles, AnglesNew, NoMovement, TotalNoMovement]=
    CollagenLocationUpdate(LocCollagen,CollagenTotal,~,~,xmin,xmax,ymin,ymax,d_norm,~,n,
    cellr)
% Compute the step amplitudes.
LocOld=LocCollagen;
```
\% Fixed radial distance to move
r=d_nrom*ones(CollagenTotal(n+1),1);

\% Compute the random step directions.
Theta=(2*pi)*rand(size(LocCollagen,1),1);
Angles=Theta;
AnglesNew=size(LocCollagen,1);

\% ThetaNew=Theta;
\% Update the locations.
\%\%x=rcos(theta), y=rsin(theta)
Loc=LocOld+[r r]*[cos(Theta) sin(Theta)];

\% Define Overcrowding
NoMovemen=[ ];

\% Boundary Conditions — no flux
\% left boundary
indx0 = find(Loc(:,1)<xmin);
[rows , \%]= size(indx0);
for j=1:rows
   Loc(indx0(j),:)=LocOld(indx0(j),:);
end

NoMovement=[NoMovement ; rows ];
TotalNoMovement=sum(NoMovement);

\% if TotalNoMovement>70
\% \% fprintf('overcrowding occurs');
\% return
\% else
\% \% fprintf('No overcrowding occurs');
\% end

\% right boundary
indxm = find(LocCollagen(:,1)>xmax);
[rows , \%]= size(indxm);
for j=1:rows
   Loc(indxm(j),:)=LocOld(indxm(j),:);
end

NoMovement=[NoMovement ; rows ];
TotalNoMovement=sum(NoMovement);

\% bottom boundary
indy0 = find(LocCollagen(:,2)<ymin);
[rows , \%]= size(indy0);
for j=1:rows
   Loc(indy0(j),:)=LocOld(indy0(j),:);
end

NoMovement=[NoMovement ; rows ];
TotalNoMovement=sum(NoMovement);

\% top boundary
indym = find(LocCollagen(:,2) > ymax);
[rows, ~] = size(indym);
for j = 1:rows
    Loc(indym(j),:) = LocOld(indym(j),:);
end
NoMovement = [NoMovement; rows];
TotalNoMovement = sum(NoMovement);

% Cell radius
indcellr = find(LocCollagen(:,2) < cellr);
[rows, ~] = size(indcellr);
for j = 1:rows
    Loc(indcellr(j),:) = LocOld(indcellr(j),:);
end
NoMovement = [NoMovement; rows];
TotalNoMovement = sum(NoMovement);
Bibliography


