Modeling Tumor-Induced Angiogenesis in the Cornea

An Honors Thesis

Presented by
Heather Harrington

Group members
Marc Maier
Lé Santha Naidoo

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Guidance Committee Approval:

Professor Nathaniel Whitaker, Mathematics

Professor Panayotis Kevrekidis, Mathematics
This work presents a new mathematical model for tumor-induced angiogenesis. We construct a continuous mathematical model based on a system of partial differential equations that describe the influences that a tumor, as well as an inhibitor, have on the growth of blood vessels in the cornea. This is a two-dimensional model that incorporates the diffusion and uptake of tumor angiogenic factors (the chemical stimuli secreted by tumors to attract cells), randomness in the rate and distance of cell growth, anastomosis (the termination of vessel formation upon intersection with a pre-existing vessel), and the presence of an inhibitor which influences cell growth in the opposite direction of the tumor. The particular novelty of this model hinges on the inclusion of the inhibitor effects and on the systematic delineation of their importance. We have developed a simulation of this biological process using MATLAB, which validates our findings qualitatively by means of comparison with previous experimental work. We have performed a sensitivity analysis on several parameters, including inhibitor strengths and diffusion rates. We first establish baseline values for all the parameters, in accordance with available experimental data, and subsequently vary the relevant values motivated by experimental assays.
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Chapter 1

Introduction

1.1 Outlook

Angiogenesis is the formation of blood vessels from a pre-existing vasculature in response to chemical stimuli. This process occurs during wound healing and embryonic development. Moreover, angiogenesis is a crucial component of tumor growth [10].

Initially, tumors do not have their own blood supply, so they depend on the diffusion of nearby blood vessels. As the tumor’s size increases, it has a greater need for a blood supply. Therefore, it must attract blood vessels to provide the necessary nourishment. These blood vessels form a network that vascularize the tumor, which promotes growth of the tumor [1]. For this reason, prevention of tumor-induced angiogenesis would be beneficial in clinical treatment of cancer. This research presents some mathematical models that describe and predict the dynamics of the angiogenesis process as influenced by a tumor.

There are many mathematical models of tumor-induced angiogenesis. Continuum models are used to understand the response of the endothelial cells to chemical and mechanical signals (through chemotaxis and haptotaxis, respectively) [1] while discrete models treat cells individually [1, 8, 17]. Discrete models are often of particular interest since they track the movements of individual sprouts and produce the morphology of vasculature networks observed in vitro. In this case,
each cell’s movements can be tracked, the processes of anastomosis and branching can additionally be modeled [1, 18].

The first model that we studied is a minimal model for tumor-induced angiogenesis [7, 8] in the body in one and two dimensions. Previous works are formulated with continuous models; however, a discrete model [1, 7, 8] is also realistic for modeling angiogenesis, as the exact position per time step is determined for the endothelial cell. This model is based on a system of partial differential equations that describes the change and movement of a cell population, extracellular matrix macromolecules, proteases, tumor angiogenic factors, and the inclusion of an inhibitor. The aim of this research is to gain familiarity with mathematical modeling in mathematical biology, and more specifically of angiogenesis with the inclusion of an inhibitor, and to produce computational experiments. To verify results, we compare the simulations to pre-existing models and experiments [4, 7, 8].

After understanding the complex processes of angiogenesis in the body, we construct a model of two-dimensional angiogenesis in the cornea of the eye based on Tong and Yuan’s earlier work [18]. We discretized the system of equations corresponding to the evolution of the TAF concentration over time, the cell’s dependence on the TAF concentration, and the cell’s direction and length of growth, as well as incorporating the possibility of new vessel growth via branching [1]. We then created simulations of this model using MATLAB, a numerical mathematics programming environment, and varied baseline values for this model performing a sensitivity analysis of the parameters in question. We qualitatively reproduced the earlier work of Tong and Yuan.
The cornea model was extended to include the effects of an inhibitor. The biology of this model is described in figure 1.1 below. This representation is consistent with the output of our simulations as seen in our results. This was done by modifying the original equations and including new equations. We monitored the results of adjusting strength, diffusion, and location (either in the vicinity or completely circumscribing the tumor) of the inhibitor. After a careful sensitivity analysis of these parameters, this model suggests a qualitative comparison to previous experiments performed by [4, 18].

![Figure 1.1: Biology of the Cornea Model](image)

Tumor-induced angiogenesis is the link between a benign tumor that can be starved to death and the more harmful vascularized tumor that can grow and allow its cells to migrate to other parts of the body to form secondary tumors. When the tumor has the ability to move to other parts of the body, it becomes harder to track and remove. Therefore, it is essential and beneficial to the tumor to vascularize in order to acquire the ability to migrate and proliferate. It is therefore relevant to identify ways to inhibit such a vascularization [10].

The aforementioned models can be employed in biological research to determine the theoretical effects of individual factors or variables influencing angiogenesis. Motivated by our findings, strategies involving manufactured inhibitors can be
Applied to regulate the angiogenesis process. The presence of an inhibitor in these models could potentially influence medical and pharmaceutical research to develop medications that prevent tumor-induced angiogenesis.

In this thesis, we will first present the biological information necessary for understanding tumor-induced angiogenesis and how it is incorporated into these models. Second, we examine a minimal model in one and two dimensions and present the results from the numerical methods [7]. The next model involves a study of Tong and Yuan’s work which emulates tumor-induced angiogenesis in the cornea [18]. Finally, we incorporate an inhibitor in the cornea model, vary parameters, and run a sensitivity analysis on this work. The results and further work will also be addressed.

1.2 Biological Processes

The initial response to starvation or oxygen deprivation from the tumor is to signal certain genes that code for signaling molecules that are used to induce angiogenesis (blood vessel formation) [10]. The molecules that are signaled are tumor angiogenic factors (TAFs), or growth factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). These TAFs activate cells that line blood vessel walls, causing them to proliferate and chemotactically attract the cells to the tumor.

Endothelial cells form the inside surfaces of blood vessels. The growth of a capillary can be tracked by the path of a single, migrating cell at the tip of the blood vessel; all other cells in the capillary follow the trajectory of the tip [17]. These cells can diffuse through interactions within the extracellular matrix (ECM) and can undergo apoptosis (cell death). The concentration gradients created by the
TAFs and the macromolecules within the ECM initiate vessel growth and influence cell movement [1]. The length of the cell growth is correlated to the strength of the concentration gradients. As endothelial cells continue to migrate and proliferate, they gradually form a vessel network within the ECM in an attempt to vascularize the tumor.

Most of the interactions involved in the angiogenesis process occur in the extracellular matrix (ECM) [8]. The ECM is the fluid in body tissue in which macromolecules and endothelial cells reside. Endothelial cells adhere to fibers in the ECM via adhesive molecules in order to gain traction to move. When TAFs are present, the endothelial cells respond by increasing the expression of the adhesive molecule receptors and then migrate to the ECM. The three main types of macromolecules that comprise the ECM are: (i) structural proteins such as collagen and elastin, (ii) specialized proteins such as fibronectin, and (iii) proteoglycans, which consist of a protein core surrounded by attached long chains of repeating units. Collagens, provide strength and elasticity to the ECM. Fibronectins are large glycoproteins which are found in basal laminae and in the loose connective tissue underneath the skin and between the body organs. In vertebrates proteoglycans occur wherever collagens occur in the ECM. Individual proteoglycans can link to collagen fibers, thereby forming the fiber-network complex of the ECM. Proteoglycans also function as sites for cell adhesions, both temporary and permanent [10].

Fibronectin functions as an adhesive between the cells and the ECM. The endothelial cells attach to fibronectin via cell surface receptors, which impedes the migration and direction of vessel growth. In addition to TAFs, proteases are also
secreted by the tumor and are located in the ECM. Proteases bind to fibronectin, which degrades the ECM, and endothelial cells no longer attach to the depleting ECM. This process contributes to the growth of the tumor as cells grow towards it with less interference.

Inhibitors also play an important role in the angiogenesis process. Inhibitors are chemicals naturally produced by the body in reaction to TAFs, but they can also be inserted artificially. When proteases are secreted by the tumor, fibroblast cells in the ECM produce inhibitors in order to inactivate the proteases [8]. Similar to TAFs, inhibitors also form a concentration gradient. This gradient repels and prevents cell growth towards the TAF, thereby limiting vascularization of the tumor. One such inhibitor is thrombospondin (TSP), a chemical widely used to deter angiogenesis [4].

Other processes associated with angiogenesis are branching and anastomosis. These processes are pictorially explained in figure 1.2 below. Branching is the generation of new vessels from the tip of a pre-existing vessel; this process is influenced by the strength of the TAF concentration [1]. Anastomosis, on the other hand, is the termination of vessel formation upon intersection with a pre-existing vessel. Together, these processes improve the networking of blood vessels and the circulation of blood.

Figure 1.2: Branching and Anastomosis
Chapter 2

Tumor-induced Angiogenesis in the Body A Minimal Model Approach

2.1 Introduction

In a preliminary effort to gain a familiarity with different aspects of the dynamics of angiogenesis and mathematical modeling, we examined a variety of published papers on tumor-induced angiogenesis and relevant mathematical models, including the earlier work of [7]. We subsequently reconstructed this work. The purpose of this exercise is five-fold: 1) achieve a thorough understanding of the biological processes involved in tumor-induced angiogenesis, 2) examine basic principles of modelling, especially in the field of mathematical biology, 3) acquire the necessary ability to read through a scientific publication and, given surface-level details presented within each paper, derive and recreate the equations and simulations in order to reproduce similar results, 4) as a direct consequence of point 3, gain an appreciation for the difficulty of constructing mathematical models via a scientific paper, and 5) acquire essential experience in converting a mathematical model into a computer program, specifically in MATLAB, in order to effectively simulate a biological process. The following section outlines our preparatory work.

The process of angiogenesis in the body involves a number of different biological factors. For the purposes of this model, we focus on the five main physical contributors, namely the endothelial cells (E), the tumor angiogenic factors, or TAFs (C), the macromolecule proteins, such as fibronectin, in the extracellular matrix
(F), the proteases (P), and the inhibitors (I). The combination of these five species and their physiological interactions form the basis for the process of tumor-induced angiogenesis. When the following five equations are solved simultaneously, they represent the continuum model of angiogenesis, in arbitrary dimensions.

\[ E_t = D_E \Delta E - \nabla \cdot (f_F \nabla F) - \nabla \cdot (f_C \nabla C) + \nabla \cdot (f_I \nabla I) + k_1 E(1 - E) \]  
(2.1)

\[ C = \exp\left[ -\frac{||x - L||}{\varepsilon} \right] \]  
(2.2)

\[ F_t = -k_2 PF \]  
(2.3)

\[ P_t = -k_3 PI + k_4 CE + k_5 C - k_6 P \]  
(2.4)

\[ I_t = -k_3 PI \]  
(2.5)

where \( f_F = a_1 E, f_C = \frac{a_2 E}{1 + a_3 C}, \) and \( f_I = a_4 E \) corresponding to the haptotactic response of cells to the adhesion gradient of the fibronectin, the chemotactic attraction of cells to the tumor, and the chemotactic repulsion of cells from the inhibitor, respectively. The previous five equations listed require a term-by-term explanation relevant to the understanding of the underlying mathematics and biology.

The primary equation, Eq. 2.1, is a representation of the endothelial cells’ evolution with respect to time. The first term incorporates simple diffusion. \( D_E \) is the diffusion coefficient and \( \Delta E \) is the laplacian, or the sum of the second spatial derivatives; together this marks the diffusion of cells from a high to a low concentration. The second term, \( \nabla \cdot (f_F \nabla F) \), signifies the haptotactic pull on the cells by fibronectin. The next term is, in principle, identical; \( \nabla \cdot (f_C \nabla C) \)
represents the chemotactic pull of the TAFs on the cells. The negative sign on these terms represents the cells’ movement toward their respective influences. The next term, $\nabla \cdot (f_I \nabla I)$, is similar to the previous two as it portrays the chemotactic repulsion by the inhibitors. As a result of the repulsion, the term has a positive sign representing the cells’ movement opposite to the driving force. The fifth and final term corresponds to apoptosis and cell generation. Natural cell proliferation is physiologically complicated; however, for the intentions of this model, a simple representation of these processes has been adopted.

In this model, we assume a static TAF concentration gradient. In actuality however, the tumor secretes TAFs that diffuse through the ECM, eventually forming a gradient. It is acceptable to consider a static TAF gradient as this minimal model represents a short time period whereas in considering models of longer time-scales, it is appropriate to consider a dynamic TAF gradient. Because of the static nature of the TAF gradient, we may ignore diffusion of TAFs and use Eq. 2.2 to represent the constant gradient. This equation is a gaussian curve centered around L, where the tumor is located. A gaussian curve resembles a bell curve where the high concentration is located at the center L, and the concentration decreases as the distance from the center increases [1].

The next equation, Eq. 2.3, describes the concentration of macromolecules in the ECM (specifically fibronectin) over time. The gradient is given a uniformly distributed random initial profile to denote the sporadic nature of the ECM. The sole term of this simple equation, $-k_2 PF$, corresponds to the depletion and degradation of the ECM by proteases.
Also secreted by the tumor are proteases, and Eq. 2.4 is a depiction of their concentration over time. The proteases have a biased, random initial profile; its distribution is multiplied by the TAF’s concentration. Incorporated into this equation are the various biological processes associated with proteases. The first term, $-k_3 PI$, models the interplay between proteases and inhibitors which act as a mutual inactivation. The next two terms, $k_4 CE + k_5 T$, relate to the increased release of proteases by the tumor and the interaction between the TAFs and endothelial cells. The last term, $-k_6 P$, depletes the concentration of proteases through natural self-inactivation.

The final equation, Eq. 2.5, models the inhibitors. The only term, $-k_3 PI$, is the same as the first term in Eq. 2.4, for the mutual neutralization of the inhibitor by the proteases. The inhibitor can be initialized in a similar fashion as the TAFs—by insertion of a gaussian curve centered at the inhibitor pellet. It is only replenished via manual insertion (achieved here by reinforcing the initial profile).

2.2 Minimal Model in One Dimension

The five species model previously mentioned describe the foundation of tumor-induced angiogenesis. These five equations explain some of the key complex biological processes associated with angiogenesis in the body. The aforementioned equations describing the cells, TAFs, extracellular matrix, proteases, and inhibitors are an idealized depiction of the physical interactions. The cells are attracted to the TAFs, which diffuse into the extracellular matrix, and to the macromolecules of the extracellular matrix. To prevent the cells from reaching the tumor, the body naturally releases inhibitors in response to tumor-induced angiogenesis. Si-
multaneously, the tumor releases proteases which neutralize the inhibitors. The continuous equations include all these terms in order to best model tumor-induced angiogenesis in the body.

To start, we consider the continuum model in one dimension. The domain is chosen to be $L = 100$ and $\epsilon$ has been chosen to be 40 (see Eq. 2.2) so that the tumor is at the center of the domain and the TAF distribution will be effective across the domain. We assume the baseline values from the works of Kevrekidis, et al. of [7] (see Table 2.1). The dimensional units of the diffusion coefficient for the cells is given as $D_E = 10^{-10} \text{cm}^2/\text{sec}$. In using the baseline values of the five species model, we are able to model the individual physiological processes; however using discrete points, as in a particle-like model, we can represent an endothelial cells’ movements with respect to time more realistically than the continuum model.

<table>
<thead>
<tr>
<th>Constant</th>
<th>Value</th>
<th>Constant</th>
<th>Value</th>
</tr>
</thead>
<tbody>
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<td>0.1</td>
<td>$a_2$</td>
<td>3.8</td>
</tr>
<tr>
<td>$a_3$</td>
<td>0.6</td>
<td>$a_4$</td>
<td>0 or 5</td>
</tr>
<tr>
<td>$k_1$</td>
<td>0.1</td>
<td>$k_2$</td>
<td>0.1</td>
</tr>
<tr>
<td>$k_3$</td>
<td>0.1</td>
<td>$k_4$</td>
<td>40</td>
</tr>
<tr>
<td>$k_5$</td>
<td>0.2</td>
<td>$k_6$</td>
<td>0</td>
</tr>
<tr>
<td>$L$</td>
<td>100</td>
<td>$\epsilon$</td>
<td>40</td>
</tr>
<tr>
<td>$D_E$</td>
<td>0.00035</td>
<td>$D_P$</td>
<td>0</td>
</tr>
<tr>
<td>$D_I$</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.1: Baseline Values of Model Constants [7]

In considering a discrete model, we must first explain certain notations. We must keep track of both temporal and spatial factors; therefore, a cell, $E$, can be expressed as discrete points in one dimension with the notation of $E_{n}^{k} = E(n\Delta x, k\Delta t)$, where $k$ is the index of time and $n$ is the spatial component of the cell. The distance between two points in the domain is $\Delta x$, and the time increment is $\Delta t$. In order to discretize partial derivatives within the equations, the
Taylor approximation is used. From this information, we found that the following notions can be used to approximate the continuous equations:

\[
\frac{\partial E}{\partial x} \approx \frac{(E_{n+1}^k - E_{n-1}^k)}{2\Delta x} \quad (2.6)
\]

\[
\frac{\partial^2 E}{\partial x^2} = \Delta_2 E^k \approx \frac{(E_{n+1}^k + E_{n-1}^k - 2E_n^k)}{\Delta x^2} \quad (2.7)
\]

Applying the same notions to all partial derivative terms in Eqs. 2.1 - 2.5, we arrive at the following set of discretized equations in one dimension:

\[
F_{n+1}^k = (1 - k_2 \Delta t P_n^k) F_n^k \quad (2.8)
\]

\[
P_{n+1}^k = (1 - k_3 \Delta t I_n^k - k_6 \Delta t) P_n^k + (k_4 \Delta t E_n^k + k_5 \Delta t) C_n^k \quad (2.9)
\]

\[
I_{n+1}^k = (1 - k_3 \Delta t P_n^k) I_n^k \quad (2.10)
\]

\[
E_{n+1}^k = P_r E_{n-1}^k + P_s E_n^k + P_l E_{n+1}^k \quad (2.11)
\]

where:

\[
P_r = \frac{D_E \Delta t}{\Delta x^2} + \frac{a_2 \Delta t}{1 + a_3 C_n^k} \left( \frac{C_{n+1}^k - C_{n-1}^k}{4\Delta x^2} \right) + a_1 \Delta t \left( \frac{F_{n+1}^k - F_{n-1}^k}{4\Delta x^2} \right) - a_4 \Delta t \left( \frac{I_{n+1}^k - I_{n-1}^k}{4\Delta x^2} \right) \quad (2.12)
\]

\[
P_s = 1 - 2D_E \frac{\Delta t}{\Delta x^2} + \left[ \frac{a_2 a_3 \Delta t (C_{n+1}^k - C_{n-1}^k)^2}{4\Delta x^2 (1 + a_3 C_n^k)^2} - \frac{a_2 \Delta t}{1 + a_3 C_n^k} \Delta_2 C_n^k \right] + a_1 \Delta t \Delta_2 E_n^k + a_4 \Delta t \Delta_2 I_n^k + k_1 \Delta t (1 - E_n^k) \quad (2.13)
\]

\[
P_l = \frac{D_E \Delta t}{\Delta x^2} - \frac{a_2 \Delta t}{1 + a_3 C_{n+1}^k} \left( \frac{C_{n+1}^k - C_{n-1}^k}{4\Delta x^2} \right) - a_1 \Delta t \left( \frac{F_{n+1}^k - F_{n-1}^k}{4\Delta x^2} \right) + a_4 \Delta t \left( \frac{I_{n+1}^k - I_{n-1}^k}{4\Delta x^2} \right) \quad (2.14)
\]

where \(\Delta_2 E_n^k\) is the discrete Laplacian, as defined in Eq. 2.7.
Now, $P_r$, $P_l$, and $P_s$ can be considered to be probabilities. They assign the relative contributions of cell movement in the next time step, $E_{n+1}^k$. $P_r$ gives the probability that the cell will move to the right in the domain. This is the contribution of $E_{n-1}^k$. Similarly, $P_l$ gives the probability that the cell moves left in the domain, the contribution from $E_{n+1}^k$. Lastly, $P_s$ is the probability that the cell will remain in the same position, $E_n^k$, which has the greatest probability. Notice that these relative weights are not true probabilities, they are appropriately normalized so the resulting probability is within $[0,1]$.

To initialize the one dimensional model, we must assign initial profiles to each of the five “species” or components. The TAFs, which remain constant throughout the simulations, maintain a gaussian curve modeled through Eq. 2.2. Fibronectin is given a uniformly distributed random profile, and the proteases receive the same, except scaled by the TAF distribution (a random profile is given to the proteases, then multiplied by the TAF distribution). The inhibitor is also assigned a gaussian curve of varying width (strength) that is discussed in the results section below.

These five equations are solved simultaneously on the same domain which reflects the true nature of these physical processes; each component has an effect on the other components. We wish to run the simulation by monitoring a single cell’s journey through the domain, keeping track of its position at every time step. Therefore, the cell’s initial profile is represented by a binary field (zero in the absence of a cell, and one if a cell exists at a particular point). For each iteration of the simulation, all equations are solved, and a uniformly distributed random number is generated between 0 and 1. This random number is used to
determine in which direction the cell will move (if at all) depending on the normalized probabilities of \( P_r \), \( P_l \), and \( P_s \).

### 2.2.1 Results and figures

Since it is primarily the effects of the inhibitor on the angiogenesis process that stimulate the most interest in this model (because of its potential relevance in clinical applications), this analysis focuses on varying properties of the inhibitor to monitor the subsequent effect on a single cell. The following results are categorized by the strength of inhibitor and the starting point of the observed cell. The tumor is centered at 80 in all of the following simulations, and the time scale is measured in iterations with each time-step \( \Delta t = 0.005 \).

![Figure 2.1](image)

(a) E at 5, no inhibitor

(b) E at 50, no inhibitor

Figure 2.1: The cell is initialized at two different positions in relation to the tumor’s center. No inhibitor is present in the domain.

### No inhibitor

The first trial has no inhibitor present. The only forces of influence on the cell are by the TAFs and fibronectin. The fibronectin adds a component of randomness in the cell’s path, but the tumor provides the ultimate attraction. Figure 2.1(a)
depicts a cell initialized at point 5, far from the tumor. The cell slowly migrates along the TAF gradient towards the greatest concentration of TAFs. Once the cell approaches \( x = 80 \), the center of the tumor and peak of TAF concentration, the cell movement reaches a plateau. The cell no longer moves in any direction, aside from random fluctuations. We now assume the cell has arrived at the tumor. Shown in figure 2.1(b) is the result of running the same simulation with the cell starting from point 50, very near the center of the tumor. The cell is immediately affected by TAF concentration and quickly arrives at the location of the tumor. Again, once there, there is only random oscillations in cell movement, but overall the cell has reached a plateau.

![Figure 2.2: The weak inhibitor temporarily delays the path of the cell toward the tumor. Once the inhibitor is diminished, the results look similar to figure 2.1.](image)

Weak inhibitor

The second set of simulations involve a weak inhibitor. The initial profile of the inhibitor is given a strength 10 times weaker than the TAF concentration. Here, we use the width of the initial gaussian curve to denote strength, not amplitude.
By having a thin gaussian, an endothelial cell is not influenced by the inhibitor’s concentration and gradient until it is close to the center of concentration. Also, for a thin gaussian curve, the concentration is depleted (by the proteases) at a faster rate than a wider area of concentration, or thicker gaussian curve. Therefore, in this model, we associate strength with the width of initial concentration. The inhibitor is not replenished in this setting, so once it has faded, it does not reappear and has no more effect on the endothelial cell’s movements. In figure 2.2(a), the cell is initialized at point 5, and the inhibitor’s center is located at 60 (from here on out). Important to note here is that the time scale of the x-axis is different from figure 2.1(a), it is precisely twice the scale because there are twice as many iterations in this simulation compared to those with no inhibitors. The weak inhibitor temporarily delays the migration of the cell, but it has a very minimal effect. The cell still progresses towards the tumor, much like if no inhibitor were present. In figure 2.2(b), the cell starts at point 50. The inhibitor has very little influence on the cell because the TAF concentration is much greater than the inhibitor. The cell is delayed only briefly, until the inhibitor concentration is diminished.

**Strong inhibitor**

A strong inhibitor, given the same initial strength as the TAFs, has a much greater influence on the migration of endothelial cells. Figure 2.3(a) shows an endothelial cell at point 5 with the strong inhibitor. The inhibitor has a tremendous effect on the cell, delaying its migration roughly 5 times as long as the weak inhibitor. The cell moves only slightly with short, local oscillations. Once the inhibitor diminishes, the cell is solely influenced by the TAF concentration. Figure
2.3(b) has the cell starting at 50, so the initial local TAF gradient is much stronger than at point 5. Now, the inhibitor still delays the cell’s progression towards the tumor, but it is not necessary for the inhibitor concentration to fade too much before the TAF concentration overwhelms the cell.

**Weak replenished inhibitor**

An important practical use of this model is to determine the necessary frequency of replenishing the inhibitor concentration to completely prevent vascularization of the tumor. Therefore, we present trials that have a fixed period of inhibitor replenishment. Every 4000 iterations, the inhibitor concentration is reinforced to its initial profile. This is analogous to manually inserting an inhibitor into the body on a timed schedule. In figure 2.4(a), the cell starts at point 5, and the inhibitor is weak, but replenished often. The cell still eventually reaches the tumor, but its progress is impeded much more than a single inhibitor as in figure 2.2(a). Qualitatively, it appears that the replenished inhibitor delays the
cell twice as long as the single inhibitor. There are small plateaux in the cells path that are observed. Each time the inhibitor concentration falls, the cell can advance towards the tumor. When the inhibitor is replenished, the cell is again delayed. This process repeats until the current inhibitor has faded and the cell reaches a strong concentration of TAF. The cell races towards the tumor before the inhibitor can counteract with its replenished concentration. Figure 2.4(b) shows the cell starting at point 50. The inhibitor has less of an effect in this simulation because the local TAF concentration is initially strong. The cell is delayed until the initial inhibitor’s concentration has faded.

**Strong replenished inhibitor**

The final simulations for the one-dimensional model involve a strong replenished inhibitor. This type of inhibitor proves to be completely effective with cells at a far distance from the tumor, see figure 2.5(a). With the cell at point 5, the TAF concentration is unable to induce vascularization against the inhibitor’s con-
concentration. Here, the inhibitor repels the endothelial cell to the boundary of the domain, with no influence by the TAFs. For the cell starting at point 50, in figure 2.5(b), the inhibitor increases the delay of the cell’s migration, but still proves to be ultimately ineffective. The TAF concentration eventually overpowers the inhibitor, allowing the cell to reach the tumor. The most practical inhibitor must have a tradeoff between strength and frequency of replenishment. A more realistic model, in terms of parameters, would allow us to quantify our results.

2.3 Minimal Model in Two Dimensions

After understanding the five species model through modeling the biological processes of tumor-induced angiogenesis in one dimension, we extended our model to include a second dimension. A two-dimensional model offers a more detailed approach to predicting and modeling angiogenesis in the body than the one-dimensional simulations. Using a two-dimensional model also presents another way to incorporate
and test the effectiveness of an inhibitor in a more realistic domain. We examine
the continuum five species equations (2.1 - 2.5) modified to include the spatial
dimensionality. In using these equations, we assume that the cells will have the
same behavior and will be attracted to the TAFs secreted by the tumor, which is
centered at \((x, y) = (80, 80)\) in the chosen domain \(L = 100 \times 100\). The cells now
have the option to move in the two-dimensional plane, meaning each cell’s position
is described by the pair \((x, y)\) as it progresses through the domain. The baseline
values of [7] are not affected by the change in dimension and can be seen in table
2.1. As in one dimension, the implementation of this model requires the discretiza-
tion of the five equations (2.1 - 2.5). The process to discretize these equations is
analogous to the one-dimensional case. The discrete model now has an additional
dimension, so our approximation of a cell’s position, \(E\), is expressed as discrete
points in two dimensions with the notation of \(E_{n,m}^k = E((n\Delta x, m\Delta y), k\Delta t)\), where
\(k\) is the index of time and \(n, m\) are the spatial components of the \(x - y\) plane. Here,
\(\Delta x\) and \(\Delta y\) are given to be the incremental distance in their respective spatial di-
rections. \(\Delta t\) is the corresponding time-step. As seen in the cell approximation
of \(E_{n}^k\), we apply the same methods of approximation, using the Taylor expansion,
for the continuous partial differential equations in two dimensions. Below are the
derived, discretized equations with the added second dimension.

\[
F_{n,m}^{k+1} = (1 - k_2\Delta tP_{n,m}^k)F_{n,m}^k
\]  
(2.15)

\[
P_{n,m}^{k+1} = (1 - k_3\Delta tI_{n,m}^k - k_4\Delta t)P_{n,m}^k + (k_4\Delta tE_{n,m}^k + k_5\Delta t)C_{n,m}^k
\]  
(2.16)

\[
I_{n,m}^{k+1} = (1 - k_3\Delta tP_{n,m}^k)I_{n,m}^k
\]  
(2.17)
\[ E_{n,m}^{k+1} = P_r E_{n-1,m}^k + P_u E_{n,m-1}^k + P_l E_{n+1,m}^k + P_d E_{n,m+1}^k + P_s E_{n,m}^k \]  

(2.18)

where:

\[ P_r = \frac{D_x \Delta t}{\Delta x^2} + \frac{a_2 \Delta t}{1 + a_3 C_{n+1,m}^k} \left( \frac{C_{n+1,m}^k - C_{n-1,m}^k}{4\Delta x^2} \right) + a_1 \Delta t \left( \frac{F_{n+1,m}^k - F_{n-1,m}^k}{4\Delta x^2} \right) - a_4 \Delta t \left( \frac{P_{n+1,m}^k - P_{n-1,m}^k}{4\Delta x^2} \right) \]  

(2.19)

\[ P_u = \frac{D_y \Delta t}{\Delta y^2} + \frac{a_2 \Delta t}{1 + a_3 C_{n,m+1}^k} \left( \frac{C_{n,m+1}^k - C_{n,m-1}^k}{4\Delta y^2} \right) + a_1 \Delta t \left( \frac{F_{n,m+1}^k - F_{n,m-1}^k}{4\Delta y^2} \right) - a_4 \Delta t \left( \frac{P_{n,m+1}^k - P_{n,m-1}^k}{4\Delta y^2} \right) \]  

(2.20)

\[ P_l = \frac{D_x \Delta t}{\Delta x^2} - \frac{a_2 \Delta t}{1 + a_3 C_{n-1,m+1}^k} \left( \frac{C_{n+1,m}^k - C_{n-1,m}^k}{4\Delta x^2} \right) - a_1 \Delta t \left( \frac{F_{n+1,m}^k - F_{n-1,m}^k}{4\Delta x^2} \right) + a_4 \Delta t \left( \frac{P_{n+1,m}^k - P_{n-1,m}^k}{4\Delta x^2} \right) \]  

(2.21)

\[ P_d = \frac{D_y \Delta t}{\Delta y^2} - \frac{a_2 \Delta t}{1 + a_3 C_{n,m+1}^k} \left( \frac{C_{n,m+1}^k - C_{n,m-1}^k}{4\Delta y^2} \right) - a_1 \Delta t \left( \frac{F_{n,m+1}^k - F_{n,m-1}^k}{4\Delta y^2} \right) + a_4 \Delta t \left( \frac{P_{n,m+1}^k - P_{n,m-1}^k}{4\Delta y^2} \right) \]  

(2.22)

\[ P_s = 1 - \frac{2D_x \Delta t}{\Delta x^2} - \frac{2D_y \Delta t}{\Delta y^2} + \frac{a_2 a_3 \Delta t}{1 + a_3 C_{n,m}^k} \left[ \frac{(C_{n+1,m}^k - C_{n-1,m}^k)^2}{4\Delta x^2} + \frac{(C_{n,m+1}^k - C_{n,m-1}^k)^2}{4\Delta y^2} \right] \]  

\[ - \frac{a_2 \Delta t}{1 + a_3 C_{n,m}^k} \Delta_2 C_{n,m}^k - a_1 \Delta t \Delta_2 F_{n,m}^k + a_4 \Delta t \Delta_2 I_{n,m}^k + k_1 \Delta t (1 - E_{n,m}^k) \]  

(2.23)

In a straightforward generalization of the the one-dimensional discretized equations, the cell in two dimensions has five possible movements in each time step: up, down, left, right, or remain in the same position. The contributed probabilities for each of these directions are reflected through \( P_u, P_d, P_l, P_r, \) and \( P_s \). In the one-dimensional case, we only consider \( P_l, P_r, \) and \( P_s \) (left, right, or same position, respectively). However, to properly describe the two-dimensional domain, we extend the range of cell movement to include the normalized probabilities, \( P_u \) and \( P_d \) (up and down, respectively).
2.3.1 Results for Two Dimensions

We simulated these modified equations to model tumor-induced angiogenesis in two dimensions. In our numerical results, we considered similar parameter variations as in the one-dimensional case. For the exclusion of an inhibitor, a weak inhibitor (replenished at time increments to ensure the inhibitor does not diffuse immediately) and a strong inhibitor, we use a domain graph and distance graph for each of these simulations. The additional dimension requires a domain graph of $x$ versus $y$ to illustrate the direction and length that the cells move, according to the normalized probabilities. The domain graph depicts how position in the $x - y$ plane changes as the cell progresses towards the tumor. The distance graph shows how the distance from the cell to the tumor changes over time. This is especially relevant for comparing and evaluating how effective an inhibitor is at preventing or slowing down the cell’s progress towards the tumor. The inhibitor, if present, is replenished every 4000 iterations.

![Domain Graph and Distance Graph](image)

(a) E at 15, no inhibitor
(b) E at 15, distance to tumor

Figure 2.6: Results for no inhibitor when the cell is far from the tumor. The left graph shows the cell growing directly towards the tumor with little deviation due to the strong attraction to the TAFs. The distance graph on the right shows that the cell reaches the tumor relatively quickly.
Figure 2.7: Results for no inhibitor when the cell is near the tumor. The cell begins to grow near the tumor at point (50, 50), and the distance graph shows that the cell moves reaches the tumor faster than the cell starting at a further distance away (see figure 2.6). The position or domain graph illustrates the cells strongly pulled by the TAFs with minimal random motion.

No inhibitor

The first trial has no inhibitor present. As in the one dimensional case, the only forces influencing the cells are the TAFs and fibronectin. The main force of attraction is provided by the TAFs, and an element of randomness is given by the fibronectin. Figure 2.6(a) depicts a cell initialized at point (15,15), far from the tumor. The cell migrates directly along the TAF gradient towards the greatest concentration of TAFs, the center of the tumor. This is clearly shown by figure 2.6(b) as we see the cell’s distance to the tumor decreasing over time.

The same trial was performed with the cell placed closer to the tumor at (50,50), as shown in figures 2.7(a) and 2.7(b). Once again, the cell is immediately attracted to the tumor and migrates along the gradient toward the largest TAF concentration. The fibronectin is partially responsible for the random movements of the cell.
(a) E at 15, weak inhibitor
(b) E at 15, distance to tumor

Figure 2.8: At a large distance from the tumor, the cell is slightly influenced by the weak inhibitor. The cell takes a longer time to reach the tumor than in figure 2.6(a)

Weak inhibitor

Next, the simulations include a weak inhibitor of strength 10 times less than that of the TAF concentration. As before, the width of the initial gaussian curve represents the strength of the inhibitor. The inhibitor’s center is located at $(x, y) = (60, 60)$.

In figure 2.8(a), the path of the cell is very similar to that of figure 2.6(a), which suggests that the cell still travels in the direction of the greatest TAF concentration. However, figure 2.8(b) shows that the inhibitor delays the cell’s migration to the tumor more than if no inhibitor were present. At certain time points in this simulation, the cell’s distance reaches a plateau, and eventually the distance to the tumor reduces to another plateau. This phenomenon is due to the rise and fall of inhibitor concentration as it diffuses and is replenished at a fixed interval.

In another realization of the same numerical experiment, the cell is initialized near the tumor at $(x, y) = (50, 50)$ (see figure 2.9(a)). Here, since the cell is placed
Figure 2.9: The cell is placed in the proximity of the inhibitor, but also near the tumor. The inhibitor is more successful with this cell placement because it delays the cell for a long period of time.

near the inhibitor, it is repelled and moves away from the inhibitor’s center. Once the inhibitor diffuses and its effect on the cell is negligible, the cell is primarily attracted by the TAF concentration and follows its gradient. Despite this pull, the inhibitor is still successful in slowing down the cell’s migration to the tumor for a longer period of time. This is shown in figure 2.9(b).

Figure 2.10: The cell is initialized far from the tumor. At this distance, the cell is closer to the inhibitor than the tumor, and therefore the inhibitor’s effects outweigh that of the TAFs. The inhibitor is very successful in delaying the cell’s progress, but does not ultimately prevent the cell from reaching the tumor.
Figure 2.11: The cell is placed in the proximity of the inhibitor, but also near the tumor. Since the inhibitor is strong (comparable to the TAF strength), it is very successful in delaying the cell’s migration towards the tumor. However, eventually the cell overcomes the inhibitor’s effects at a time when the concentration is low and has not yet been replenished.

**Strong inhibitor**

The simulation was also performed with a strong inhibitor which has the same initial strength as the TAFs. This inhibitor has a much greater influence on the cell than the previous weak inhibitor. Figure 2.10(a) shows the migration of the cell in this scenario. Again, we see the inhibitor’s initial repulsion of the cell followed by the TAFs influence towards the tumor. However, figure 2.10(b) shows that the inhibitor successfully delays the cell’s movement because it withholds the cell at a greater distance for a longer time period than that of the weak inhibitor.

Figure 2.11(a) shows the cell placed close to the tumor. The cell is initially in the proximity of the inhibitor and, therefore, realizes its effects immediately. On the other hand, the TAF concentration is also significant at this point. Even though the migration is towards the TAFs, the inhibitor still keeps the cell away from the tumor for a longer time (see figure 2.11(b)) in comparison to the cell placed further from the tumor (see figure 2.10(b)).
Chapter 3

Cornea Model

3.1 Introduction

Having completed the minimal model of angiogenesis in the body, we now proceed to study more specific models of angiogenesis. In particular, we model tumor-induced angiogenesis in the cornea. As a starting point, we examined and explored all aspects of the research and model of Tong and Yuan [18] in order to understand the biological processes and equations of this model and to contrast it with the minimal model [8]. Particularly, the appealing features of the cornea remain in its two-dimensional nature. Due to its small size, there are fewer proteins that interact in the cornea and, therefore, make the biological processes slightly different than those in the entire body. Also included in this model are the processes of branching, formation of new vessels from the tips of pre-existing vessels, as well as anastomosis, the cell death incurred by intersection with a pre-existing vessel.

To understand this complicated process, we studied the specific interactions among angiogenic factors and vascular networks. Forming a comprehensive and realistic model requires a thorough biological examination, followed by a mathematical investigation consistent with experimental assays and data. The resulting models can either be continuous or discrete, depending on the level of description. Continuous models describe angiogenesis based on density changes in blood vessels; however, vascular networking and growth of individual vessels can be mon-
monitored in discrete models [1, 17]. We examined the model of Tong and Yuan to grasp the differences between their model and pre-existing ones; in comparison to experimental results, Tong and Yuan suggest that this particular model is an adequate representation of the modeled physical processes [18]. This is a two-dimensional model which is physiologically realistic taking into account the thin structure of the cornea. The model includes the growth of vascular networking of blood vessels, and the TAF concentration gradient is dynamic, i.e., it changes over time [7]. We choose the cornea model based on the nature of the cornea’s geometry, which makes simulations easier. Additionally, the rat cornea has been used in other studies related to angiogenesis [4, 9] and can be used to obtain experimental data and directly compare with our model.

3.2 Processes, Biology of Cornea and Explanation of Model

As previously mentioned, the biology of the cornea is inherently simple. The cornea is the small, transparent surface that forms the eye’s outermost layer. It is an avascular membrane, which means that there are no blood vessels within it, unlike other tissues throughout the rest of the human body. The cornea relies on tears and the aqueous humor located behind it to receive its nourishment [12]. The closest vasculature to the cornea is a limbal vessel at the junction of the cornea and sclera of the eyeball. A limbal vessel is a blood vessel acting as the border or edge of a body part, see figure 1.1. Therefore, to model angiogenesis in the cornea, the only biological factors that we must consider are the TAFs and endothelial cells. The TAFs are secreted by a tumor within the cornea, and the source of endothelial cells is the pre-existing limbal vessel surrounding the cornea. In this model, the angiogenic factors secreted by a tumor within the cornea initiate the growth of
blood vessels, starting at the limbal vessel along the edge of the cornea, towards the tumor. The absence of other factors, such as proteases and fibronectin, is a direct result of the avascular nature of the cornea [7]. The ECM in this model is assumed to be negligible, having a weak response to the spread of angiogenic factors and the growth of blood vessels. Inhibitors are not considered in Tong and Yuan’s model; the following chapter deals with our extension of this model, specifically concerning the inclusion of inhibitors.

The model is simplified by the fact that the cornea can be assumed to be well approximated by a circular disk of radius 3mm, roughly equivalent to the area of a rat cornea [18]. The full domain of our simulation matches that of Tong and Yuan, 8mm by 8mm, large enough to accommodate wide-spread TAF diffusion.

### 3.3 Equations

The principal dynamical features of TAFs in the cornea are 1) diffusion within the extracellular matrix of the cornea 2) the natural inactivation of TAFs 3) the uptake of TAF by cells. These factors are incorporated into the following equation.

\[
\frac{\partial C}{\partial t} = D_c \left( \frac{\partial^2 C}{\partial x^2} + \frac{\partial^2 C}{\partial y^2} \right) - kC - uLC
\]

Tong and Yuan address the problem that concentration gradients are not independent of time [17, 1]. The first term in Eq. 3.1 describes TAF diffusion using the standard Laplacian operator, involving the sum of the spatial second derivatives. The diffusion coefficient, \(D_c\), influences the rate of the diffusion of the TAFs from the tumor or a manually inserted TAF pellet. The second term, \(-kC\), symbolizes the natural inactivation of the TAFs, with an inactivation constant, \(k\), which is
proportional to the TAF concentration, $C$. The last term that governs the dynamics of the TAFs, $-uLC$, models the uptake of the TAFs by the endothelial cells, where $L$ denotes the density of cells at a local point. If there is a cell present at this discrete point, then the cell will naturally uptake the TAF concentration at a rate constant, $u$; moreover, this uptake term is also proportional to the TAF concentration.

We assume the concentration of the TAFs is fixed at the location of the tumor [18]. The tumor constantly releases TAFs so that TAFs are at a maximum concentration at the site of the tumor. The TAF concentration is normalized to range from 0 to 1, where a concentration of 1 represents the TAFs near the tumor, and 0 represents a negligible concentration. The assumption that the TAF concentration remains constant in the proximity of the tumor is important when considering simulations since the concentration will appear to be ever-present to the cells.

An endothelial cell must recognize tumor angiogenic factors in order to be influenced or attracted towards the tumor. Additionally, the TAF concentration value, $(C)$, must be greater than a threshold value, $C_t$, which is the minimum TAF concentration needed to have impact on endothelial cell growth. If the concentration of the TAFs is below this threshold value, then a cell feels no pull or attraction. This is demonstrated in the threshold function of Eq. 3.2. If the concentration is above $C_t$, then an endothelial cell recognizes the presence of the concentration gradient, which produces an attraction towards the TAF gradient.
The dependence of cells on the concentration of the TAFs may be modeled using the following threshold function.

\[
f(C) = \begin{cases} 
0, & 0 \leq C < C_t; \\
1 - \exp[-\alpha(C - C_t)], & C_t \leq C.
\end{cases}
\]  

(3.2)

Our model utilizes the threshold function in branching, a biological process that generates a new blood vessel from a pre-existing vessel ultimately resulting in two blood vessels. Since the biology of the cornea is such that there is no pre-existing vasculature in the cornea, there is a given probability that a vessel will sprout from the established limbal vessel. In Eq. 3.3, \( \bar{n} \) represents the probability of branching by the TAF concentration threshold function, \( f(C) \). This probability is also dependent on \( S_{\text{max}} \), the constant coefficient of maximum branching. The other two terms that influence the branching are the total length of the vessel, \( \Delta l \), and the time increment, \( \Delta t \).

\[
\bar{n} = S_{\text{max}} f(C) \Delta l \Delta t
\]  

(3.3)

When endothelial cells grow, the direction of growth varies at every time step; therefore, the direction of growth must be determined every time a cell moves. The equation that models direction permits the cell to move in any direction rather than the minimal model’s limitations of discrete up, down, left, or right movements. As seen in Eq. 3.4, the direction of vessel growth \( (E_x, E_y)^T \) is found by taking a combination of the cell’s previous movements, \( (E_x^0, E_y^0)^T \), and the direction of the TAF gradient, \( (G_x^0, G_y^0)^T \). A persistence ratio, \( P \), is introduced here to give weights to each of these components and additional preference to
the cell’s previous movement. Finally, a rotational matrix is multiplied by the entire direction of sprout growth equation to provide randomness in the direction of cell growth. The cell can wander randomly (as in a random walk) from the predicted direction, which is influenced by the TAFs and previous movement, due to structural changes of the ECM dynamics [18]. From Tong and Yuan, we assume the angle of deviation, $\theta$, to be a between $\pi/2$ and $-\pi/2$, with $\tan \theta$ having a gaussian distribution with mean 0 and variance $\sigma = .5$.

$$
\begin{pmatrix}
E_x \\
E_y
\end{pmatrix}^T = \left\{ P \begin{pmatrix}
E^0_x \\
E^0_y
\end{pmatrix}^T + (1 - P) \begin{pmatrix}
C^0_x \\
C^0_y
\end{pmatrix}^T \right\} \cdot \begin{pmatrix}
\cos \theta & \sin \theta \\
-\sin \theta & \cos \theta
\end{pmatrix} \quad (3.4)
$$

As mentioned before, the model incorporates the realistic process of cells moving various distances or lengths at each time iteration. The calculation of a cell’s length at each time movement is contingent on the maximum rate of length increase, $V_{\text{max}}$, and on the concentration threshold of TAF, $f(C)$. The combination of these two factors results in the growth rate of the cells as modeled through the following equation.

$$
\Delta l = V_{\text{max}} f(C) \Delta t \quad (3.5)
$$

This rate of growth $V_{\text{max}}$ along with the localized concentration of the TAF, $f(C)$, and the interval of time, $\Delta t$, are used to obtain the length increase of cells, $\Delta l$. The three previous factors model the proliferation of cells, the attraction to the TAFs and the speed that the cell grows during a given time interval.
The last biological process of importance is anastomosis, which is the process of two blood vessels merging into one. At every iteration each cell is checked to determine its location relative to all other vessels. If the cell is within a certain local proximity of another vessel, its growth terminates and a single sprout emerges thereafter.

3.4 Baseline Values

In order to qualitatively compare and validate our model against Tong and Yuan’s, we used the baseline values of constants that were presented in their paper. Keeping this in mind, the TAF in our simulations was basic fibroblast growth factor (bFGF) [8], a TAF that is commonly studied in tumor angiogenesis. Baseline values used in this model are shown in Table 3.4.

<table>
<thead>
<tr>
<th>Constant</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffusion coefficient of bFGF, $D_c$</td>
<td>$0.5 \times 10^{-6} \text{cm}^2 \text{s}^{-1}$</td>
</tr>
<tr>
<td>Rate constant of bFGF degradation, $k$</td>
<td>$2.89 \times 10^{-2} \text{h}^{-1}$</td>
</tr>
<tr>
<td>Rate constant of uptake, $u$</td>
<td>$2000 \mu \text{m} \text{h}^{-1}$</td>
</tr>
<tr>
<td>Shape constant in the threshold function, $\alpha$</td>
<td>10</td>
</tr>
<tr>
<td>Threshold concentration, $C_t$</td>
<td>.001</td>
</tr>
<tr>
<td>Persistence ratio, $P$</td>
<td>0.5</td>
</tr>
<tr>
<td>Variance of deviation angle, $\sigma$</td>
<td>0.5</td>
</tr>
<tr>
<td>Maximum rate of sprout length increase, $V_{\text{max}}$</td>
<td>$20 \mu \text{m} \text{h}^{-1}$</td>
</tr>
<tr>
<td>Rate constant of sprout formations, $S_{\text{max}}$</td>
<td>$5 \times 10^{-4} \mu \text{m} \text{h}^{-1}$</td>
</tr>
</tbody>
</table>

Table 3.1: Baseline Values of Model Constants [18]

$D_c$ was calculated by [18] based on the molecular weight of bFGF and an equation by [2].

The rate constant, $k$, represents the natural degradation of bFGF per hour. The exact half-life of bFGF is unknown, but it varies between a few hours and 24 hours. [18] found that fixing the degradation rate based on a half-life between 5 and 24 hours does not significantly change the density and patterns of vascular
networks, as long as the other baseline values are held constant. For this reason, $2.89 \times 10^{-2} h^{-1}$ is used as the value of $k$.

It is known that bFGF induces angiogenesis, and the effects of bFGF are proportional to the concentration of the TAFs, to a certain extent. The threshold concentration, $C_t$, is the minimum TAF concentration needed to influence angiogenesis. On the other hand, the saturation point is the maximum concentration at which the TAFs have no “pull” on vessels. [3] found that when the amount of TAFs was increased from 30 to 3000 $ng/ml$, the vessel density was augmented proportional to this rise of TAF concentration. On the other hand, when the amount of TAF was changed from 3000 to 30,000 $ng/ml$, the density of vessels did not increase. This shows that the saturation point is at least 10 times smaller than the threshold point. Tong and Yuan state that it is more reasonable for the order of magnitude to be between two and four times smaller than the saturation point, but the TAF concentration used in the model is twice that of the saturation point. Based on these assumptions, $\alpha$ was chosen to be 10 and $C_t$ to be 0.1% (or 0.001) of unbound TAF concentration in the pellet.

The maximum rate of vessel growth has been chosen to be $20 \mu m/h$. It has been shown that endothelial cells at the tips of vascular networks move at a speed of $15 \mu m/h$ in response to bFGF stimulation. In addition, endothelial cells migrate in vitro at an approximate speed of $20 \mu m/h$. However it is worth noting that when acidic fibroblast growth factor (aFGF) is added, the speed increases to about $40 \mu m/h$.

The estimated rate of sprout branching is based on experimental data. The data showed that in a 3 day period, 15 sprouts of $0.88 mm$ were connected to
previously existing vasculatures. It was assumed that half of these vessels were new branches, whereas the other half were vessels that intersected with the pre-existing vessel, undergoing anastomosis. From this data, we see the average sprout formation is approximately $10^{-4} \mu m^{-1} h^{-1}$. Tong and Yuan arbitrarily assumed that the maximum rate of formation, $S_{max}$, was five times the average and found this value to be reasonable through their sensitivity analysis of variables.

The persistence ratio, $P$, and the variance of deviation angle, $\sigma$, could not be estimated from experimental data. Tong and Yuan arbitrarily chose these values to be 0.5.

### 3.5 Simulation Details

This model is unique due to the “hybrid” aspect of the domain, where we use both continuous and discrete variables. In other words, the domain is half discretized and half real-valued. Normally, a discretized field consists of a mesh of discrete points; however, in this model, a single vessel grows as if the domain were a continuous, real-valued plane. The diffusion of TAFs occurs on the discrete grid, but the vessels grow over the entire domain. A cell can grow in any direction for a real-valued distance. In many other discrete models, such as the minimal model [8] discussed previously, it is assumed that a cell can move in a finite number of directions at a fixed length. The concept of a “hybrid” domain generates a number of complications, the most prominent of which is the additional time required to complete the computer simulations. For example, the minimal model could run on the order of a few hours; the cornea model is on the order of days, approaching a whole week. This complexity hinges on the anastomosis implementation. Given that a vessel could be at any position in the domain, it is necessary to consider
the exact coordinates of all other cells, as well as where those cells have been to in the past. This is done in order to determine whether or not the cell in question is within a certain proximity to another existing vessel. If so, anastomosis ensues, resulting in the termination of the cell. This check is performed every time step for each cell. Unfortunately, the simulation consists of thousands of iterations, in which there could be several hundred cells.

### 3.6 Results

After successfully interpreting Tong and Yuan’s earlier work and model and subsequently transforming the underlying mathematics into a computer program in MATLAB, it was necessary to compare our results to those given in their paper. Figures 3.1(a) and 3.1(b) are the results of running the baseline simulation as depicted in Tong and Yuan’s paper. These figures qualitatively match Tong and Yuan’s simulations. The novelty of this model rests on several factors. The cornea is one of the only body parts which has a circular shape. This changes the dynamics of tumor-induced angiogenesis in that the vessels are restricted to a different domain. This model also includes the processes of branching and anastomosis in order to establish a more realistic simulation of angiogenesis.

Tong and Yuan varied many constants and parameters to understand individual effects on the model. These variations consistently matched experimental observations and demonstrate that the patterns of a vascular network in the presence of a tumor is dependent on the concentration of the TAFs in the given environment, as well as the distribution of its gradient. It is also clear that the vessel growth is time dependent. As time progresses and the TAFs diffuse, the
growth of the vessels gradually increase, and the probability of branching rises as the parent vessel ages and approaches the tumor.

One of the factors affecting the diffusion of the TAFs is the uptake by endothelial cells. Tong and Yuan ran simulations in which the rate constant of uptake, $u$, was changed to see the effects on sprout formation and growth. Their model showed that if $u$ is decreased (by a factor of 5), formation and growth of vessels accelerated and there was an increased occurrence of self loops (parent and child vessels that anastomose with each other). When $u$ was increased by a factor of 5, Tong and Yuan found that vessel formation and growth was retarded, and branching did not occur as frequently.

The direction of vessel growth is modeled through Eq. 3.4. The angle of deviation, $\theta$, adds an element of randomness to the movement of vessels. In the model, $\theta$ is random, and the magnitude of randomness is determined by the variance of $\tan(\theta)$, $\sigma$. With a high $\sigma$, there is a lot of variation in the vessels’ movement with less emphasis on moving towards the tumor. The randomness leads to a greater occurrence of anastomosis and vessel loop formation. It was also observed that when the vessels approach the vicinity of the tumor, self loops form more rapidly and frequently, to the benefit of the tumor (a better vascular network for added blood flow is formed). When $\sigma$ is lowered, there is very little randomness in vessel movement, and as a result, vessels move directly towards the tumor with little deviation from its path. This vascular structure is not beneficial to the tumor because it limits the nutrition supply. The vascular network with self loops provide more vessel area to vascularize the tumor. In the body, the amount of randomness is a result of the surrounding environment.
$S_{\text{max}}$, the rate constant of sprout formation, also influences vessel growth. By raising $S_{\text{max}}$ a factor of 5, the number of vessels in the cornea increases, but it decreased the average length of the vessels. This generates a brush border, an extremely dense population of vessels in the proximity of the tumor. When $S_{\text{max}}$ is lowered by a factor of 5, there is no brush border, and the number of vessels decreases.

The model presented by Tong and Yuan, which we interpreted and reproduced, is a good representation of the processes involved in angiogenesis in the cornea. They provide a good qualitative analog to physical processes, and it can be used for future studies and extensions.

![Figure 3.1: No inhibitor results](image)

(a) zoomed in  
(b) TAF gradient

Figure 3.1: No inhibitor results
Chapter 4

Cornea Model Extended to Include an Inhibitor

4.1 Introduction

An extension of Tong and Yuan that would be particularly relevant to applications consists of the inclusion of an inhibitor in the cornea model. The added effects of an inhibitor can be used in researching defenses against tumor-induced angiogenesis. As seen in the minimal model [8], an inhibitor is a chemical that repels the direction of cell growth. The minimal model serves as a way to understand how the inhibitors, when secreted in the presence of TAFs, can fight off tumor-induced angiogenesis in the cornea. In this particular model, we used experimental data [4] in order to contrast our simulations with physical in vivo results. Specifically, thrombospondin (TSP) is a widely used inhibitor that deters angiogenesis [5]. TSP can be used as a chemical pellet which is manually inserted into the cornea. In our model, we test the effectiveness of the inhibitor based on where it is positioned, through testing representative examples, such as the case where the inhibitor is either circumscribing or in the near proximity of the tumor.

It is relevant to include an inhibitor in the cornea model because the growth of endothelial cells towards the tumor contributes to the vascularization of the tumor, which in turn leads to pathological effects on the body. We know from previous models [7, 8, 9] that an inhibitor is a key factor in the prevention of tumor-induced angiogenesis. In other models, the effects of an inhibitor are known to
slow down the angiogenesis process. This is accomplished by the inhibitor repelling endothelial cell growth. Therefore, if the inhibitor is placed in a position that will maximize the repulsion from itself and the tumor, the vascularization of the tumor will take longer in the presence of such an inhibitor. In addition, if the inhibitor is sufficiently strong, it is possible to prevent angiogenesis from occurring at all. The addition of an inhibitor to the cornea model has the potential of providing medical and cancer research with a better and more insightful way to prevent tumor-induced angiogenesis. The inhibitor types, diffusivities, and strengths play important roles in determining whether or not an inhibitor is effective in deterring angiogenesis. In order to consider the inclusion of an inhibitor, we first studied the effects of TSP and examined how it interacts in the cornea with the endothelial cells and TAFs. Using this information, we were able to define the inhibitor’s role specific to our benchmark model for angiogenesis in the cornea. We also added and modified the equations of Tong and Yuan’s cornea model [18] to match the biology underlying the relevant/corresponding processes. Once our new model was established, a thorough sensitivity analysis was performed to verify baseline values and determine the precise effects of the different parameters in our model, especially of the ones concerning the inhibitor.

4.2 Equations

When including an inhibitor to the model, we must take into account the effects its gradient will have on the TAFs, as well as endothelial cell growth and movement. The extent to which the inhibitor affects its surrounding environment is dependent upon its concentration and diffusion rate through the ECM. The change in the inhibitor concentration over time depends on its natural diffusion and certain
depleting effects caused by presence of TAFs. The dynamic inhibitor is modeled through the following equation:

\[
\frac{\partial I}{\partial t} = D_I \cdot \left( \frac{\partial^2 I}{\partial x^2} + \frac{\partial^2 I}{\partial y^2} \right) - k_{on} IC \quad (4.1)
\]

The first term in the equation above, \( D_I \cdot \left( \frac{\partial^2 I}{\partial x^2} + \frac{\partial^2 I}{\partial y^2} \right) \), is the sum of the second spatial derivatives, also called the Laplacian, and represents the diffusion of the inhibitor gradient from a high to low concentration. The diffusion coefficient, \( D_I \), scales the rate of the inhibitor pellet in the simulation to match that of a manually inserted pellet in the body. The second term in the equation, \(-k_{on} IC\), represents the interplay between the inhibitor and TAFs. The \( k_{on} \) coefficient represents the rate constant of inhibitor depletion caused by the TAFs.

The interplay term is also included into the equation modeling the TAF concentration depletion. This term is again negative as the TAF and inhibitor mutually work to annihilate the other. Eq. 3.1 for the TAFs now becomes:

\[
\frac{\partial C}{\partial t} = D_C \cdot \left( \frac{\partial^2 C}{\partial x^2} + \frac{\partial^2 C}{\partial y^2} \right) - kC - uLC - k_{on} IC \quad (4.2)
\]

The inhibitor is given an initial condition, that is designed to emulate the artificial insertion of an inhibitor. This concentration diffuses and degrades up to a predetermined time interval. Once the inhibitor is depleted, it is replenished to the initial inhibitor concentration profile. This concentration is the maximum possible concentration unless altered by changing the strength of the inserted pellet. The concentration around the inhibitor pellet varies based on the diffusion
of the inhibitor pellet and its distance from the source. Baseline values for this
model are shown in Table 4.1.

<table>
<thead>
<tr>
<th>Constant</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffusion coefficient of bFGF, $D_c$</td>
<td>$0.5 \times 10^{-6} \text{cm}^2\text{s}^{-1}$</td>
</tr>
<tr>
<td>Rate constant of bFGF degradation, $k$</td>
<td>$2.89 \times 10^{-2}\text{h}^{-1}$</td>
</tr>
<tr>
<td>Rate constant of uptake, $u$</td>
<td>$2000\mu\text{mh}^{-1}$</td>
</tr>
<tr>
<td>Interplay constant, $k_{on}$</td>
<td>$3.45 \times 60\text{M}^{-1}\text{hr}^{-1}$</td>
</tr>
<tr>
<td>Diffusion coefficient of inhibitor, $D_I$</td>
<td>$10^{-6}\text{cm}^2\text{s}^{-1}$</td>
</tr>
<tr>
<td>Shape constant in the threshold function, $\alpha$</td>
<td>10</td>
</tr>
<tr>
<td>Threshold concentration of TAFs, $C_t$</td>
<td>0.001</td>
</tr>
<tr>
<td>Threshold concentration of inhibitor, $I_t$</td>
<td>0.001</td>
</tr>
<tr>
<td>Initial inhibitor strength</td>
<td>10</td>
</tr>
<tr>
<td>Persistence ratio, $P$</td>
<td>0.6</td>
</tr>
<tr>
<td>Variance of deviation angle, $\sigma$</td>
<td>0.5</td>
</tr>
<tr>
<td>Maximum rate of sprout length increase, $V_{max}$</td>
<td>$20\mu\text{mh}^{-1}$</td>
</tr>
<tr>
<td>Rate constant of sprout formations, $S_{max}$</td>
<td>$5 \times 10^{-4}\mu\text{mh}^{-1}$</td>
</tr>
</tbody>
</table>

Table 4.1: Baseline Values of Model Constants

In order for an endothelial cell to be influenced by the TAFs and the inhibitor,
their concentration must be greater than a certain critical value. Similarly, the
inhibitor must also be above a certain threshold concentration in order for it to
have an effect on the endothelial cells. This threshold concentration is denoted
as $I_t$. Therefore, as seen in Eq. 4.3, if the inhibitor concentration is less than
$I_t$, then the gradient value is 0 and the endothelial cell does not recognize the
presence of the inhibitor. If the inhibitor concentration is greater than the thresh-
hold concentration, the value given from the threshold function is some value up
to 1; where 1 corresponds to the highest concentration levels. The values of the
gradient are given by a threshold function and are used as a scaling mechanism in
other equations in the model. The threshold function for the inhibitor is similar
to that of the TAFs (as shown in the previous chapter) and is as follows:

\[ f(I) = \begin{cases} 
0, & 0 \leq I < I_t; \\
1 - \exp(-\alpha(I - I_t)), & I_t \leq I.
\end{cases} \]  

(4.3)

The values attained from the threshold function are present in the equations that determine the probability of branching, change in vessel length, and the direction of vessel growth.

As previously mentioned, branching is the process of blood vessel generation from pre-existing vessels. It is also known that TAFs promote blood vessel growth, and inhibitors repel cells. We have also shown that the probability of branching by the TAF concentration is shown by \( \pi \) in Eq. 3.3. Now, we include the inhibitor’s (negative) contribution to branching, \( \overline{m} \):

\[ \overline{m} = -S_{\text{max}} f(I) \Delta l \Delta t \]  

(4.4)

Just as in \( \pi \), the probability is dependent on the coefficient of the maximum probability of sprouting, \( S_{\text{max}} \), the inhibitor threshold function, \( f(I) \), the total length of the vessel from the start of growth, \( \Delta l \), and the time increment, \( \Delta t \). The negative sign in this equation denotes the negative effect that the inhibitor has on vessel branching contrary to what is the case of the TAFs.

The combined effect of the TAF and inhibitor on branching has been taken to be the maximum of \( \pi + \overline{m} \) and 0. By doing this, if \( \pi \) is greater than \( \overline{m} \), the endothelial cell will have a (positive) probability of branching; whereas, if \( \overline{m} \) is greater, then the probability will be 0, and no branching will occur.
The direction of vessel growth changes every time step. Previously, it was noted that the direction of growth was determined by the endothelial cell’s previous direction of motion, the TAF concentration gradient, and a rotational matrix that incorporates a random element of growth with the persistence ratio, $P$, giving weights to each component. Once the inhibitor is included, it also influences the direction of growth of vessels, but in the opposite direction of itself and essentially the TAFs. This is reflected in the following modifications to the prior direction equation, Eq. 3.4:

\[
\begin{pmatrix}
E_x \\
E_y
\end{pmatrix}^T = P \begin{pmatrix}
E_0^x \\
E_0^y
\end{pmatrix}^T + \frac{(1-P)}{2} f(C) \begin{pmatrix}
G_0^x \\
G_0^y
\end{pmatrix}^T - \frac{(1-P)}{2} f(I) \begin{pmatrix}
I_0^x \\
I_0^y
\end{pmatrix}^T.
\]

\[
(4.5)
\]

In this equation, Eq. 4.5, the direction of vessel growth, $(E_x, E_y)^T$, is found by taking a combination of the cell’s previous movements, $(E_0^x, E_0^y)^T$, the direction of the TAF gradient, $(G_0^x, G_0^y)^T$, and the direction of the inhibitor gradient, $(I_0^x, I_0^y)^T$. The persistence ratio, $P$, is shared among each component with most weight given to the previous direction of movement and equal weights given to the TAF and inhibitor gradients. The rotation matrix remains the same as in the previous model [18].

The distance that the endothelial cells move also varies at each time step. This distance is measured by the maximum velocity, $V_{max}$, multiplied by the time increment, $\Delta t$, with the weight of the concentration gradients also contributing.
respectively to the vessel growth. The absolute value of the TAF and inhibitor concentrations are taken to ensure growth instead of death, and the difference is taken to incorporate the opposite effects of the TAF and the inhibitor. The change in vessel length is shown as:

$$\Delta l = V_{max} \mid f(C) - f(I) \mid \Delta t$$  \hspace{1cm} (4.6)

The equations mentioned above model the blood vessels movement and interactions in the ECM in the presence of an inhibitor. It is clear that the inhibitor affects the TAF gradient, as its purpose is to counteract it so that the tumor will be deprived of nutrients (vascularization will not occur) and ultimately die. The equations show the conflicting effects of the tumor and the inhibitor in the equations regarding endothelial cell movement. The next section illustrates our findings through a sensitivity analysis of the relevant parameters.

4.3 Results and Discussion

To better understand our model, as well as to confirm the baseline values chosen, we performed an extensive sensitivity analysis on many of our parameters. In this section we present the results of our analysis and discuss our interpretation of how each variable impacts the outcome of the model. We start by introducing the results of our simulations in accordance with the established baseline values. There are two separate trials involving the baseline values: the circumscribing inhibitor (the inhibitor partially surrounds the tumor) and the geometric inhibitor (the inhibitor placed at a certain distance away from the tumor). The next several
sections detail the analysis performed on specific variables, including inhibitor diffusion, inhibitor strength, interplay between inhibitor and TAFs, and persistence ratio.

### 4.3.1 Baseline Values

As mentioned above, two of our simulations focus around the baseline values. In the first trial, the inhibitor is placed at a distance from the tumor in order to observe the geometric effects that the inhibitor has on the model, especially the networks of vessels. At the end of simulation, here 50,000 iterations or approximately 30 hours, the inhibitor has repelled vessels away from its gradient. The cornea at this stage is shown in figure 4.1(a), and the inhibitor gradient is seen in figure 4.1(b).

The dynamic inhibitor depletes due to its natural diffusion and the effects of the TAFs and is therefore periodically replenished, in this case every 800 iterations. Because of this depletion, at certain times the vessels can traverse through the inhibitor. When the inhibitor has diminished, the vessels recognize its presence and are able to pass through the corners of the inhibitor pellet. The vessels do not directly cut through the center of the pellet, but rather the corners, where the inhibitor gradient is weakest in comparison with the TAF gradient. This illustrates the effectiveness of the inhibitor’s repulsion on the vessels. Figure 4.1(b) shows a snapshot of the inhibitor gradient halfway through a replenished interval.

Comparing this gradient to figure 4.1(c), the TAF gradient, it is quite apparent that the TAF gradient is the dominating force of the two. The TAF gradient does not diminish; in fact, there is a constant source behind the TAF gradient, namely the tumor, which reinforces the angiogenic factors every iteration. This critical
The difference between the TAFs and the inhibitor is the main reason that the blood vessels travel towards the tumor, with only a slight detour around the inhibitor.

\[ \text{sech} \left| x - x_0 \right| \cdot \left| y - y_0 \right| \]

The second simulation that strictly uses baseline values has an inhibitor partially circumscribing the tumor. Unlike the geometric inhibitor, the initial profile of this inhibitor uses the hyperbolic secant and incorporates a metric of absolute value distance. The function is \( \text{sech} \left| x - x_0 \right| \cdot \left| y - y_0 \right| \), and the gradient resembles a castle (shown in figure 4.2(a)). We say that the inhibitor partially circumscribes the tumor because the norm used in its describing function does not make use of absolute values instead of Euclidian distance. Therefore, the gradient is not the typical “volcano,” but rather the “castle” of figure 4.2(a). If the Euclidian distance had been used, then the inhibitor would be circular in shape.
and could completely circumscribe the tumor. Here, however, the inhibitor has a diamond-like shape that overlaps the tumor (seen in figure 4.2(b)).

Through the course of this simulation, which only required 15,000 iterations or approximately 9 hours, the blood vessels grow directly towards the tumor. There are no impediments along the path of any vessel because they do not perceive the inhibitor until they are in close proximity of the tumor (see figure 4.2(b)). However, as soon as the vessels approach the south edge of the tumor, they are influenced by the inhibitor gradient. The same results are magnified in figure 4.2(c) to better visualize the effects of the inhibitor. The vessels, although strongly attracted to the tumor, avoid any areas covered by the inhibitor gradient. The vessels appear to slightly change the direction of their paths in order to circumvent the inhibitor. This is exactly what was expected. If the tumor was entirely circumscribed by the inhibitor and replenished at a sufficient rate, the blood vessels could principally be completely prevented from vascularizing the tumor.

An interesting aspect of this simulation worth noting is the TAF gradient (see figure 4.2(d)). In the areas of the gradient that overlap with the inhibitor gradient, the TAFs have diminished. This is a direct effect of the interplay term in the TAF diffusion equation of Eq. 4.2.

4.3.2 Inhibitor Diffusion

Another parameter considered is the diffusion rate of the inhibitor which changes the speed that the inhibitor gradient travels throughout the cornea. The diffusion influences the cell growth due to the large or small amount of surface area the gradient covers. It is expected that an inhibitor with high diffusivity will reach a further distance enabling the repulsion of cell growth earlier in the simulation. In
the first simulation, the diffusion is five times greater than the baseline value, see figure 4.3(a). When the stronger diffusion is compared to the baseline figure, it is clear that the endothelial cell growth starts; however, it ceases very quickly due to the diffusion of the inhibitor concentration gradient. The negative effect the inhibitor has on blood vessel growth occurs almost immediately, which makes this inhibitor quite efficient. Notice that the cells that normally grow near the inhibitor do not even begin to sprout. Another aspect of the higher diffusion can be seen in the inhibitor gradient (figure 4.3(b)) where the base of the gradient is wider and covers a large distance of the cornea domain. The TAF profile (figure 4.3(c)) appears to have rather jagged cuts into the entire side; therefore, due to the high diffusion of the inhibitor gradient, it heavily diminishes the TAF concentration.
When running simulations on diffusion coefficients, we must also explore an inhibitor that has smaller diffusivity than the baseline inhibitor. High diffusivity decreased blood vessel growth, but a lower diffusion rate is expected to lower the influence or effect on cell growth. As seen in figure 4.4(a), the distance the inhibitor profile covers is less due to the diffusion being ten times less than the baseline diffusion rate. Since there is less diffusivity, the vessels are weakly influenced by the inhibitor. The inhibitor concentration (figure 4.4(b)) base width is much narrower than the baseline, and this is noticeable compared to the higher diffusion of figure 4.3(b). Another simulation of an even weaker diffusion rate shows that the lower diffusion, the less influence it has on cell repulsion, as seen in figure 4.4(c).
4.3.3 Inhibitor Strength

In conducting our analysis, we found that varying the strength of the inhibitor matched our initial predictions. The strength of the inhibitor gradient does not change the distance the gradient covers as does the diffusion, but it creates a higher “wall” of concentration. The concentration increases so that as blood vessels recognize the inhibitor via the threshold function, the blood vessels are repelled immediately and at a greater distance. Essentially, the concentration of the inhibitor is replenished at higher values, which makes the inhibitor more potent. This strong inhibitor repels the cells much more than the baseline (see figure 4.5(a)); the strength of the inhibitor in this simulation is five times greater
than the baseline model. Also, notice the inhibitor pellet is completely avoided by the blood vessels. Due to this impediment on the cells, it takes a longer time period for the vessels to vascularize the tumor. To demonstrate the effects of a stronger inhibitor, see the enhanced figure 4.5(b). The impact of a stronger inhibitor provides an explanation for the TAF gradient’s slice through its center in figure 4.5(d). This is a result of the interplay reaction between the inhibitor and TAF gradients. This shows why the cells are repelled at a greater distance around the inhibitor in this simulation.

Figure 4.5: Inhibitor strength at 50 results

4.3.4 TAF and Inhibitor Interplay - $K_{on}$

When certain chemicals come into contact with each other, a chemical reaction occurs. Such is the case between the TAFs (bFGF) and the inhibitor (TSP). This
reaction degrades both the TAF concentration and the inhibitor concentration, controlled by the coefficient $K_{on}$. The term $-K_{on}IC$ is present in Eq. 4.1 and Eq. 4.2. This models the feature that the concentration of the TAFs and inhibitor is affected because of the chemical interaction between the two. The value for $K_{on}$ was derived through calculations described in [11] and applied to Figure 3 in [5]. The baseline value for $K_{on}$ was chosen to be $3.45 \times 60M^{-1}hr^{-1}$. The units of $K_{on}$ are in $M^{-1}hr^{-1}$ in order to resolve the $M^2$ from the product of the TAF and inhibitor concentrations.

Both the TAF gradient and inhibitor gradient are equally affected since the degradation is proportional to both concentrations. A small $K_{on}$ value will allow for the persistence of the inhibitor gradient, but a large value will reduce its concentration as well as the TAFs. Since the inhibitor is local to a specific area of the cornea domain and the TAF gradient is only affected in the presence of the inhibitor, the TAFs are depleted locally. This is shown in figures 4.6(c) and 4.7(d). With the small value of $K_{on}$ (5 times less than the baseline), the TAF concentration has minimal change. Similar results occur on the inhibitor concentrations. For small $K_{on}$, the interplay between TAF and inhibitor is less, so the inhibitor can grow and diffuse more, see figure 4.6(d). In the case of the large value of $K_{on}$ (5 times greater than the baseline), the TAFs are drastically reduced within the locale of the inhibitor and the inhibitor is degraded by the TAFs. The inhibitor gradient becomes very thin with a low peak, figure 4.7(c).

Further discussion is necessary to elucidate the effect of the phenomenon just described, on the blood vessels. Given the equations above for vessel length and direction of growth, the initial results did not qualitatively match our predictions.
In figure 4.6(a), the vessels that approach the tumor tend to terminate while all other vessels grow normally. One would expect that the vessels would be repelled by the inhibitor as the inhibitor now has a more diffused gradient with a small $K_{on}$; however, the vessels that approached the inhibitor appeared to stop their growth. The central reason behind this is a direct result of Eq. 4.6. In this equation, the length of vessel increase is governed by the difference of the two threshold functions, $f(C)$ and $f(I)$. The problem occurs when these two quantities are roughly equal, which causes the vessel to grow at an extremely small length. This should principally hold if the tumor and inhibitor were in the same location (as in the circumscribing inhibitor scenario), but, in this case, the vessel should grow normally with a large length. This effect should occur through the direction
of vessel growth, which is properly calculated through Eq. 4.5, but it does not exhibit the proper effect since the length of vessel growth is negligible. Therefore, the vessel length equation was modified as follows to model this result:

$$\Delta l = V_{\text{max}} \|f(C) \begin{pmatrix} G^0_x \\ G^0_y \end{pmatrix} - f(I) \begin{pmatrix} I^0_x \\ I^0_y \end{pmatrix} \| \Delta t$$ (4.7)

This equation incorporates the norm of the difference between the two gradient direction vectors of TAF and inhibitor, scaled by their respective threshold functions. The norm is the Euclidian distance, and this value approaches zero only if the two vectors oppose each other, which incorporates the correct physical effect.

Figure 4.7: $K_{\text{on}}$ at 1035
Redoing the simulations with this updated equation, the results appear to be considerably more physical. For small $K_{on}$, the TAF is not degraded near the inhibitor as much, so the vessels follow the TAF gradient. However, in the updated simulation, the vessels do circumvent the inhibitor pellet (except for the vessels at the bottom-left of the pellet where the two gradient vectors are opposite each other, as anticipated), see figure 4.6(b). For large $K_{on}$, the inhibitor has a greater effect on the TAFs. Therefore, the vessels primarily see the inhibitor gradient and, even though there is less concentration of inhibitor, the blood vessels are repelled to a much greater distance. The vessels travel around the inhibitor until the TAF gradient dominates and pulls them on the path towards the tumor, see figure 4.7(a). The difference before and after the modification of Eq. 4.7, in this case, is minimal (see figures 4.7(a) and 4.7(b)).

**4.3.5 Persistence Ratio**

As mentioned earlier the persistence ratio, $P$, included in the equation modeling the direction of vessel growth is used to assign relative weights to the contributing factors of vessel growth direction. The larger the value for $P$, the more emphasis is placed on increasing growth in the direction of the vessels previous movement. This allows a preference for the inertial effects of vessel growth. If this persistence ratio is changed, the path of the vessels are altered as the weights are distributed differently among the influencing factors. Below, in figures 4.8(a) and 4.8(b), we see an increase in the persistence ratio from our baseline, 0.6, to 0.8. This increase places an added emphasis on the vessel’s natural movement from the previous time step rather than the influence by the TAF or inhibitor gradient. Due to
in this favoring, the vessels have a more tortuous structure, due to the increase in variability over directly moving away from the inhibitor or towards the tumor.

(a) Angiogenesis with large persistence ration

(b) Zoomed in

Figure 4.8: P at 0.8 results
Chapter 5

Conclusions

In this work, we presented two different models of tumor-induced angiogenesis. These models were the minimal model of angiogenesis in one and two dimensions and the model of angiogenesis in the cornea with and without an inhibitor.

The minimal model, based on the works of Kevrekidis, et. al. [7, 8], presents a basic incorporation of a number of key the biological processes of angiogenesis and interactions in the body between the endothelial cells, TAFs, fibronectin, proteases, and inhibitors through five partial differential equations. When these equations are solved simultaneously, they produce a continuum “5 species” model. The drawback of this continuum model is that the individual movements of the endothelial cells cannot be tracked. In order to accurately portray vessel networks based on the chemical environment of the ECM, the model is discretized.

It is clear that the inhibitor plays a role in delaying angiogenesis by diverting cells away from itself. If the inhibitor is significantly strong or is replenished at an appropriate frequency, it could possibly prevent angiogenesis from occurring, thereby potentially starving the tumor of essential nutrients. Simulations were done both in one and two dimensions. This minimal model appears to qualitatively match experiments done by D.J. Good et. al. [4].

The model of angiogenesis in the cornea is a two-dimensional representation of a discretized system of equations corresponding to chemical changes in the
TAFs and its influences on endothelial cell movement and growth. Branching and anastomosis are also represented in this model. The particular differences between this model and the minimal model lies in the circular shape and the two-dimensional nature of the domain. The model also incorporates a dynamic profile of the TAF concentration. This model, based on the work of S. Tong and F. Yuan, qualitatively match the results presented by Tong and Yuan.

The model of the cornea was then extended to include an inhibitor. In doing so, the equations of the previous model were modified and other equations were added in order to take into account the inhibitor’s effects on the endothelial cells and TAFs. We ran trials with different inhibitor concentrations and orientations to determine which would be the best placement of the inhibitor to prevent the vessels from reaching the tumor. As in the minimal model, it was found that the inhibitor, depending on its strength and placement, can influence the endothelial cells enough to prevent it from reaching the tumor. A sensitivity analysis was performed to observe the individual effects of certain constants and coefficients used in the model. It was also noted that the inhibitor’s success is dependent on the interplay term between the tumor and the inhibitor, as well as the diffusion coefficient of the inhibitor.

Both of the above mentioned models can further be extended to include a third dimension. This will model angiogenesis more realistically, as the body contains three dimensions. The minimal model can also be changed to show the proliferation and migration of multiple cells rather than a single particle, and the cornea model can be modified to add the effects of other “species,” such as proteases in the cornea.
These models are useful because they provide a qualitative representation of angiogenesis that can be used in studies towards a more quantitative understanding. Individual factors can be monitored and varied in order to observe the individual effects of these factors. The models can also be used for a clearer comprehension of the length and time scales of angiogenesis and can therefore be used to devise a strategy for inhibiting angiogenesis and to generate experimentally testable hypotheses.
Bibliography


