A hybrid model for tumor-induced angiogenesis in the cornea in the presence of inhibitors

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Abstract

The present work formulates and analyzes, by means of numerical experiments, a model for tumor-induced angiogenesis in the presence of inhibitors in the cornea. Our model is a generalization of the earlier work of Tong and Yuan [S. Tong, F. Yuan, Numerical simulations of angiogenesis in the cornea, Microvascular Research 61 (2001) 14–27] to incorporate the role of inhibitors, relevant to experimental assays. The derived set of hybrid equations consists of partial differential equations for the tumor angiogenic factors and the inhibitors with a particle model for the motion of the endothelial cells. This is analyzed numerically in the two-dimensional setting. The relevant results are discussed and qualitative agreement with the experimental work is illustrated.

Keywords: Angiogenesis; Inhibitor; Vascular network; bFGF

1. Introduction

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 (see e.g. [2] and references therein). Moreover, angiogenesis is a crucial component of tumor growth [9]. Initially, tumors are not vascularized (i.e., they do not have a network of blood vessels surrounding them to supply nutrients); therefore, they rely on the proliferation of proximate vessels. To promote angiogenesis, a tumor secretes tumor angiogenic factors (TAFs), such as the basic Fibroblast Growth Factor (bFGF), that attract endothelial cells to form new capillary sprouts. Inhibitors also play an important role in the angiogenesis process. Inhibitors are chemicals naturally produced by the body in response to TAFs under normal conditions, or can be artificially inserted. These prevent cell growth towards the TAF, thereby limiting vascularization of the tumor. One such inhibitor is thrombospondin-1 (TSP), which is widely used to deter angiogenesis [3]. Interactions between the TAFs, the inhibitor, and the endothelial cells occur in the extracellular matrix (ECM) [5], the fluid in body tissue in which macromolecules and endothelial cells reside.

The cornea is the small, transparent surface that forms the eye’s outermost layer. It is an avascular membrane, 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 [11]. The closest vasculature to the cornea is a limbal vessel at the junction of

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the cornea and the sclera of the eyeball, see Fig. 1. Identifying ways to prevent tumor-induced angiogenesis would be useful in clinical treatment of cancer [9]. In this context, the study of an avascular tissue such as the cornea is rather beneficial, as one can identify key mechanisms contributing to the angiogenic process and directly compare them to available experimental results [3]. A more specific goal of this direction of research is to illustrate mechanisms through which angiogenesis can be prevented, thereby resulting in tumor starvation and subsequent necrosis. Our efforts will be focused on this direction, using the presence/insertion of an inhibitor in an effort to completely deter vascularization of the tumor. The effectiveness of an inhibitor can be tested through a mathematical model that describes the relevant processes, which we will develop herein.

Naturally, the importance of the above research theme has driven a large volume of recent research towards the direction of understanding the process of angiogenesis. There are two principal classes of models used in this effort, namely continuum and discrete ones. Continuum models are used to understand the response of the endothelial cells to chemical and mechanical signals [2,7] while discrete models treat cells individually [2,5,13,1]; see also the detailed recent review of [9]. 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. This desirable feature of the latter setting has also driven efforts to construct hybrid models, where the endothelial cells are described in a discrete form, while other quantities (such as the TAFs, the inhibitors, or the extracellular matrix constituents, such as fibronectin) are described by continuum concentrations governed by partial differential equations [2,6,5,14].

The work presented herein falls under the umbrella of hybrid models. More specifically, we extend the two-dimensional discrete model of angiogenesis in the cornea proposed by [14] by adding the physiologically (and experimentally [3]) relevant situation of the natural presence, or artificial insertion, of an inhibitor. In our model, we examine the effects of the inhibitor based on its position (highlighting interesting geometrical effects in the angiogenic process) and other controlling factors, including inhibitor strength and diffusivity. The underlying mathematics behind the model consists of a system of partial differential equations that represent the evolution of TAFs and inhibitor concentrations, coupled to the discrete dynamics of the cells and the formation of cell vascular networks.

Our paper is structured as follows: we first introduce the details of the mathematical model and its relation to the aforementioned biology (Section 2). Next, we analyze our numerical results in Section 3 and conclude with a discussion and summary of this model and possible future extensions in Section 4.

2. Model and setup

The motivation for the present work, as highlighted above, was the earlier discrete model of Tong and Yuan [14] in conjunction with the relevant experiments in the cornea in the presence of thrombospondin (operating as an inhibitor) of [3]. In the former theoretical effort, the authors investigated the principles of angiogenesis in the cornea, including a dynamic TAF concentration, cell migration induced by tumor angiogenic factors, branching of vessels, and anastomosis (vessel termination upon intersection with pre-existing vasculature). Our aim in emulating more closely the experimental work of [3] is then to integrate an inhibitor in this framework. The role of the latter is to operate as an “opposing factor” toward the chemotactic attraction of endothelial cells to TAFs, in an effort to delay and potentially completely prevent angiogenesis.
A rather reasonable assumption for our purposes is to approximate the cornea by a circular disk of diameter 6 mm, as is roughly the case for the rat cornea [14]. The full domain of our simulation is 8 mm × 8 mm.

The principal equations of the model are the partial differential equations (PDEs) for the dynamical space-time evolution of the TAF and inhibitor concentrations. The extent to which the TAFs \((C = C(x, y, t))\) and inhibitor \((I = I(x, y, t))\) affect their surrounding environment is dependent on their concentrations and diffusion rates in the ECM. The TAF concentration is modeled through the PDE:

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

The features of TAFs in the cornea that are incorporated in the model are the following (in the order that they appear in the right hand side of Eq. (1)):

- diffusion within the extracellular matrix of the cornea with diffusivity \(D_C\);
- the natural inactivation of TAFs (with rate \(k\));
- the uptake of TAFs by cells with rate constant \(u\); \(L\) here denotes the local density of cells controlled by the presence \((L = 1)\) or absence \((L = 0)\) of cells;
- and, finally, the interplay term between the inhibitor and TAFs, resulting in the mutual inactivation of TAFs and inhibitors with rate constant \(k_{on}\).

Interestingly, one of the most challenging tasks is to identify experimentally relevant estimates for the rate constant \(k_{on}\). Such a value was derived through calculations described in [10] and applied to Fig. 3 in [4]. The baseline value for \(k_{on}\) was chosen to be \(3.45 \times 60 \text{ M}^{-1} \text{ h}^{-1}\) in the relevant dimensional units (for details see [4,10]). Similar to the TAFs, the local inhibitor concentration also depends on the inhibitor diffusivity, \(D_I\), within the extracellular matrix (ECM) and the depleting effects caused by the mutual inactivation of inhibitors and TAFs. Our equations simplify the actual direct interaction of this biological process. The relevant PDE then reads:

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

Finally, we discuss the discrete part of the model pertaining to the proliferation of the endothelial cells. An endothelial cell must recognize the presence of tumor angiogenic factors or an inhibitor in order to be influenced by their respective concentrations. Following [14], we impose a threshold value, \(C_t\), i.e., a minimum TAF concentration necessary for endothelial cell growth to be affected by TAFs. If the concentration of the TAFs is below this threshold, then a cell does not perceive the TAF chemical signaling. However, if the concentration is above \(C_t\), then an endothelial cell detects the concentration gradient and is influenced accordingly. The inhibitor affects cell growth in a similar manner with threshold concentration \(I_t\). This is demonstrated as follows in Eqs. (3) and (4):

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

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

\(\alpha\) controls the shape of the curve; in particular, for larger \(\alpha\), the shape of the response resembles a piecewise constant function, with a sharp transition near the critical concentration \(C_t\); on the other hand, for smaller \(\alpha\), the response depends more sensitively on the actual concentration (beyond the critical concentration \(C_t\)). In principle, the parameter \(\alpha\) need not coincide between the TAF and inhibitor responses, but for the sake of simplicity and since the response is of the same type (i.e., chemotactic), we have selected the two shape-determining coefficients to be identical. Due to the lack of experimental data, the functions above were constructed based on a previous study of position-dependent pattern formation in embryonic development [8]. Following [14], the value \(\alpha = 10\) is used in Eq. (3). Due to the lack of data, the same value for \(\alpha\) is also used for the inhibitor in Eq. (4). However, these values can be different.

In order for an endothelial cell to proliferate, two main factors should be considered: the direction and length of vessel growth. Here, we allow the direction of growth to be affected by three factors: (1) the endothelial cell’s
previous direction of motion, (2) the TAF concentration gradient, and (3) the inhibitor concentration gradient. A rotational matrix incorporates the possibility for a random growth direction. This is reflected in the following direction equation:

\[
\begin{pmatrix} E_x \\ E_y \end{pmatrix}^T = \left\{ P \left( \begin{pmatrix} E_x^0 \\ E_y^0 \end{pmatrix}^T + \frac{(1 - P)}{2} f(C) \left( \begin{pmatrix} C_x^0 \\ C_y^0 \end{pmatrix}^T - \frac{(1 - P)}{2} f(I) \left( \begin{pmatrix} I_x^0 \\ I_y^0 \end{pmatrix}^T \right) \right) \right\} \cdot \begin{pmatrix} \cos \theta & -\sin \theta \\ \sin \theta & \cos \theta \end{pmatrix}.
\] (5)

The direction of vessel growth \((E_x, E_y)\) is found by taking a combination of the cell’s previous movements, \((E_x^0, E_y^0)\), the direction of the highest TAF concentration, \((C_x^0, C_y^0)\), and the direction of the highest inhibitor concentration, \((I_x^0, I_y^0)\). A persistence ratio, \(P\), gives relative weights to each contributing factor (see details in the next section). Finally, a rotational matrix provides for the possibility of randomness in the direction of cell growth, implicitly incorporating the potential for structural changes of the ECM dynamics [14]. Following Tong and Yuan, we assume the angle of deviation, \(\theta\), to be an angle between \(-\pi/2\) and \(\pi/2\), with \(\tan \theta\) having a Gaussian distribution with mean 0 and variance \(\sigma = 0.5\).

Since each tube consists of several endothelial cells, we define the increase in vascular tube length, \(\Delta l\), as:

\[
\Delta l = V_{\text{max}} \left\| f(C) \begin{pmatrix} C_x^0 \\ C_y^0 \end{pmatrix} \Delta t - f(I) \begin{pmatrix} I_x^0 \\ I_y^0 \end{pmatrix} \right\| \Delta t.
\] (6)

The maximum velocity of length increase, \(V_{\text{max}}\), is multiplied by a time increment, \(\Delta t\), to provide the extension of the vascular structure. This equation also incorporates the norm of the difference between the two direction vectors of the TAFs and the inhibitor scaled by their respective threshold functions. Doing so allows the endothelial cell growth to incorporate the strength and direction of the pull/push by the TAFs/inhibitors (respectively) to the cells and to appropriately incorporate the balance of the relevant factors.

Branching, which leads to the generation of new blood vessels from pre-existing vessels, is an important process in tumor-induced angiogenesis [2]. In order to provide optimal blood flow to the tumor, a complex network of vessels is necessary. Since the biology of the cornea is such that there is no pre-existing vasculature, there must be a given probability that a vessel will sprout from the established limbal vessel. This probability is also used to determine the rate of branching once a vessel is in motion. The combined effect of the TAF and inhibitor on branching is taken to be \(\max (\bar{\pi} + \bar{m}, 0)\), where

\[
\bar{\pi} = S_{\text{max}} f(C) \Delta l \Delta t
\] (7)

\[
\bar{m} = -S_{\text{max}} f(I) \Delta l \Delta t.
\] (8)

The probability that a vessel will branch is dependent on the coefficient of maximum sprouting rate, \(S_{\text{max}}\), the threshold functions, \(f(C)\) and \(f(I)\), the total length of the vessel from the start of growth, \(\Delta l\), and the time increment, \(\Delta t\). It is known that TAFs promote branching while inhibitors reduce cell growth; therefore, the negative sign in Eq. (8) denotes the “negative” effect that the inhibitor has on vessel branching.

The baseline values applied to this model are mostly obtained from [14]. Specifically, we assume identical values as [14] for the constants \(k, u, \sigma, D_C, \alpha, C_I\) and \(V_{\text{max}}\) (the interested reader is directed to that work for the justification of the relevant parameter values). The present model additionally incorporates certain new and modified parameters related to the inclusion of the inhibitor. The diffusion coefficient, \(D_I\), for the inhibitor was based on experimental assays on thrombospondin-1 (TSP) performed by [3]. This value was calculated to be \(10^{-6}\) cm² s⁻¹. The rate constant, \(k_{on}\), as previously described, was deduced to acquire a typical value of \(3.45 \times 60\) M⁻¹ h⁻¹ (see the discussion above). We also assumed that the threshold value for the inhibitor, \(I_t\), was 0.001. While somewhat arbitrary, this choice (similar to that of [14] for the TAFs) has no significant bearing on the main numerical findings of the paper. The persistence ratio, \(P\), was modified to allow for the presence of the inhibitor. \(P\) was typically given a value of 0.6, indicating an “inertial” preference to the cell’s previous direction of motion. The rate constant of sprout formation, \(S_{\text{max}}\), has the value of \(25 \times 10^{-4}\) μm h⁻¹. Table 1 summarizes all baseline values used in this model.

We assume the concentration of the TAFs is fixed at the location of the tumor [14]. The tumor is placed within the cornea and is uniformly initialized to a unit value throughout the pellet (see also Fig. 1). Similarly, the inhibitor is given an initial condition designed to emulate an artificially inserted inhibitor. The strength of the inhibitor pellet is initially set to ten times the strength of the TAFs. Notice that while the tumor provides a constant source of TAFs,
Table 1
Baseline values of model constants

<table>
<thead>
<tr>
<th>Description</th>
<th>Constant</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffusion coefficient of bFGF</td>
<td>$D_C$</td>
<td>$0.5 \times 10^{-6}$ cm$^2$s$^{-1}$</td>
</tr>
<tr>
<td>Rate constant of bFGF degradation</td>
<td>$k$</td>
<td>$2.89 \times 10^{-2}$ h$^{-1}$</td>
</tr>
<tr>
<td>Rate constant of uptake</td>
<td>$u$</td>
<td>$2000$ µm h$^{-1}$</td>
</tr>
<tr>
<td>Interplay constant</td>
<td>$k_{on}$</td>
<td>$3.45 \times 60$ M$^{-1}$ h$^{-1}$</td>
</tr>
<tr>
<td>Diffusion coefficient of inhibitor</td>
<td>$D_I$</td>
<td>$10^{-6}$ cm$^2$s$^{-1}$</td>
</tr>
<tr>
<td>Shape constant in the threshold function</td>
<td>$\alpha$</td>
<td>$10$</td>
</tr>
<tr>
<td>Threshold concentration of TAFs</td>
<td>$C_t$</td>
<td>$0.001$</td>
</tr>
<tr>
<td>Threshold concentration of inhibitor</td>
<td>$I_t$</td>
<td>$0.001$</td>
</tr>
<tr>
<td>Initial inhibitor strength</td>
<td>$P$</td>
<td>$0.6$</td>
</tr>
<tr>
<td>Persistence ratio</td>
<td>$\sigma$</td>
<td>$0.5$</td>
</tr>
<tr>
<td>Variance of deviation angle</td>
<td>$V_{max}$</td>
<td>$20$ µm h$^{-1}$</td>
</tr>
<tr>
<td>Maximum rate of sprout length increase</td>
<td>$S_{max}$</td>
<td>$25 \times 10^{-4}$ µm h$^{-1}$</td>
</tr>
</tbody>
</table>

The diminishment of the inhibitor concentration necessitates replenishment over regular time intervals. This fixed interval varies based on inhibitor characteristics, such as placement and strength.

3. Numerical results and discussion

We start by exploring the model phenomenology for the set of baseline values discussed in the previous sections. Subsequently, we perform a sensitivity analysis on several parameters that influence the inhibitor in order to analyze the impact each has on the outcome of the model. The analysis was performed on specific aspects of the model. We examine, in particular, the inhibitor placement, inhibitor diffusion, inhibitor strength, interplay between inhibitor and TAFs, and persistence ratio.

3.1. Baseline values

The inhibitor is initially placed at a distance from the tumor in order to observe geometric effects of the inhibitor. At the end time (30 h) of the simulation the inhibitor has repelled vessels away from its gradient. The cornea at this stage is shown in Fig. 2(a). In all figures, the inhibitor pellet is represented by the small rectangle with the light border and the tumor is represented by the larger rectangle with the darker border. Inhibitor concentration is seen in Fig. 2(b) halfway through a replenishment interval (see details below about the replenishment of the inhibitor). The dynamic inhibitor diffuses as well as depletes due to its interaction with the TAFs. This effect occasionally leads the vessels to traverse through the corners of the inhibitor pellet, where the inhibitor effect is weakest in comparison with the one of the TAFs. It is apparent that the TAF gradient is the dominating force among the two, see Fig. 2(c). We assume here that the tumor supplies a constant source of TAFs. This is the principal reason that the blood vessels travel towards the tumor, using pathways with a slight “detour” around the inhibitor.

In our model, we replenish the inhibitor concentration every 30 min for the inhibitor placed away from the tumor. In contrast, for a circumscribing inhibitor, the replenishment must occur more often (approximately every 3.33 min) in order to prevent the vessels from reaching the tumor. This increase in frequency balances the more rapid depletion of the inhibitor concentration caused by the TAFs (see the discussion on TAF and inhibitor interplay below).

Notice that there is very little brush bordering (formation of very dense vascular networks) in these simulations. The number of vessels initialized is relatively small. In addition, the probability of branching is bounded above as determined by Eqs. (7) and (8). The inhibitor decreases this probability and, therefore, reduces the possibility of a brush border effect. This differs from [2], which has a branching probability approaching 1 in the vicinity of the tumor. Our model is primarily concerned with the behavior and properties of the inhibitor.
3.2. Inhibitor placement

The second simulation we show also uses baseline values but has an inhibitor partially circumscribing the tumor. Unlike the previous case, the initial profile of this inhibitor is of the form

\[
I(x, y, t = 0) = \text{sech}(|x - x_0| \cdot |y - y_0|),
\]

(9)

see Fig. 3(c).

In this case, through the course of this simulation, the blood vessels grow directly towards the tumor. There are no impediments along the path of any vessel since the cells do not perceive the inhibitor until they are in close proximity of the tumor, see Fig. 3(a) and (b). However, as soon as the vessels approach the south edge of the tumor, they are influenced by the inhibitor gradient. These results are magnified in Fig. 3(b) to better visualize the effects of the inhibitor. The vessels, although strongly attracted to the tumor, avoid any areas covered by the inhibitor by slightly changing the direction of their paths in order to circumvent the inhibitor. An interesting observation here is that if the tumor was entirely circumscribed by the inhibitor and replenished at a sufficient rate, then the blood vessels could, in principle, be completely prevented from vascularizing the tumor. Fig. 3(d) shows the direct effect of the interplay term in the TAF diffusion equation of Eq. (1). Notice that in areas of overlap between the TAFs and the inhibitor, the TAF concentration gets depleted.

3.3. Inhibitor diffusion

The diffusion rate of the inhibitor is another important factor that affects the angiogenesis process of endothelial cells. Since inhibitor diffusion can be influenced by many components in the ECM, which are not considered in this
model, we assume for simplicity it diffuses uniformly. An inhibitor with high diffusivity will spread further within the domain, enabling the repulsion of cell growth earlier in the simulation. In the first example shown below, the diffusivity is five times greater than the baseline value, see Fig. 4(a). The adverse effect that the inhibitor has on blood vessel growth is considerably stronger in this case (see also the discussion below about the number of vessels in this case). An inhibitor with higher diffusion disperses to farther distances throughout the domain than the baseline inhibitor. As a result, the inhibitor concentration is larger throughout the domain while the TAF concentration is accordingly depleted which causes the TAFs to be less influential on the endothelial cell direction of motion.

An inhibitor with a smaller diffusivity than the baseline inhibitor was also considered. A lower diffusion rate (0.5 times less than the baseline) decreases the influence on cell growth. Fig. 4(b) shows the relevant evolution dynamics. Because the small diffusivity reduces the area of spread of the inhibitor concentration, the blood vessels have the opportunity to travel closer to the inhibitor pellet than in the baseline scenario. Therefore, the vessels can traverse through the corners of the inhibitor pellet during intervals of low inhibitor concentration.

3.4. Inhibitor strength

We have also modified the potency of the inhibitor by increasing the magnitude of the initial profile by a factor of 5. Such an increase may occur by administering higher doses of an artificial inhibitor in experiments similar to those performed in [3]. A visible consequence of this variation is that the blood vessels appear to be repelled at a greater distance. This effect is shown in Fig. 5(a) and (b). The inhibitor pellet is completely circumvented by all blood vessels.
Fig. 4. Inhibitors of varying diffusivity: Fig. (a) incorporates a diffusion coefficient of 5 times the baseline. The vessels react strongly to this largely diffused inhibitor concentration and are greatly repelled. The smaller diffusivity, 0.5 times less than the baseline, has the opposite effect, as shown in (b). The vessels easily reach the tumor with minimal deviation caused by the inhibitor.

Fig. 5. Inhibitor strength results. An increase in inhibitor strength to 50 illustrates the repelling effect of the inhibitor. Vessels are repelled at a greater distance than in the baseline case.

Due to this impediment on the cells, it takes a longer time period for the vessels to vascularize the tumor. We should also note in passing that the stronger effect on the TAF concentration from the inhibitor results in a large “hole” in the TAF profile (in the location of the inhibitor pellet).

3.5. TAF and inhibitor interplay — $k_{on}$

The effect of the interaction between the TAFs and the inhibitor is controlled by the interplay constant, $k_{on}$, as was also mentioned previously. A small $k_{on}$ value will allow for the persistence of the inhibitor concentration, but a large value tends to considerably reduce its concentration as well as that of the TAFs. Since the inhibitor is local to a specific area of the corneal domain and the TAF concentration is only affected in the presence of the inhibitor, the TAFs are depleted locally. With the small value of $k_{on}$ (5 times less than the baseline), both the inhibitor and TAF concentrations have minimal change. 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.

Further discussion is necessary to elucidate the effect of the phenomenon just described on the blood vessels. For small $k_{on}$, the TAF concentration is minimally degraded near the inhibitor, hence the vessels follow the TAF gradient. The vessels circumvent the inhibitor pellet, except for the vessels at the bottom-left of the pellet where the two gradient vectors are equal and opposite each other, see Fig. 6(a). For large $k_{on}$, the inhibitor has a greater effect on the TAFs. Therefore, the vessels primarily recognize the inhibitor concentration and, even though there is less inhibitor, the
4. (a) Angiogenesis with small \( k_{on} = 41.4 \).

(b) Angiogenesis with large \( k_{on} = 1035 \).

Fig. 6. \( k_{on} \) variation results. A small \( k_{on} \) value in (a) promotes minimal depletion effects of the TAFs on the inhibitor, and vice versa, therefore allowing each to perform its respective “job” with equal efficiency. However, a large \( k_{on} \) value in (b) promotes great TAF depletion in the proximity of the inhibitor, and vice versa. This therefore magnifies the efficacy of the TAFs and inhibitor in their respective locales.

3.6. Persistence ratio

As previously mentioned, 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 of \( P \), 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. Below, in Fig. 7(a) and (b), we monitor the results of an increase in the persistence ratio from our baseline, 0.6, to 0.8. This increase places an additional emphasis on the vessel’s inertial movement from the previous time step rather than the influence of the TAF or inhibitor gradient. Consequently, the resulting vasculature has a more tortuous structure.

3.7. A quantitative measure: Vessel number

The basic (qualitative) phenomenology of the model has been adequately represented in the sensitivity analysis presented above. However, to give a more quantitative measure of the observed behavior, in Fig. 8, the number of endothelial cells (vessels) is shown over time. In the baseline case, the cells proliferate at a linear rate but the branching
Fig. 8. Vessel statistics (number of vessels vs. time). In (a), the baseline case, cells proliferate at a linear rate until branching saturates. (b) demonstrates the effect of inhibitor placement. The rate of branching and the time vessels take to approach the tumor are less than the baseline case. The effects of inhibitor diffusivity are shown in (c) and (d). Low diffusivity does not differ from (a) except that no saturation of branching occurs; high diffusivity spreads the inhibitor concentration throughout the domain causing no branching at all. A small $k_{on}$ value causes cells to proliferate at a slower rate than the baseline (see (e)), and a large $k_{on}$ value (see (f)) increases the rate of cell growth, thereby resulting in a later branching saturation point than that of the baseline case. In (g), a stronger inhibitor clearly reduces the rate of branching.

eventually saturates. The vessel sprouts are also slowed by the diffusion of the inhibitor, especially in the vicinity of the inhibitor pellet. In this case, the cells near the inhibitor take approximately 17–19.5 h to start reaching the tumor, whereas cells that are further away from the inhibitor arrive in the vicinity of the tumor from about 5.5 h. When the placement of the inhibitor is changed to circumscribe the tumor, the rate of branching and the time vessels take to approach the tumor are less than the baseline case. With this placement, it appears that vascularization of the tumor by the cells is delayed. The cells in the proximity of the inhibitor start reaching the tumor after around 14 h and the
cells that are closest to the tumor arrive after, approximately, 11–13 h. There are timescale differences between these simulations and those of [14]. A typical simulation of [14] was 4 days, whereas our evolution span is approximately 30 h. The number of vessels initialized in the domain is considerably smaller than in [14], and is a factor for this discrepancy.

The effects of inhibitor diffusivity were analyzed at a magnitude of 0.5 times less than and 5 times greater than the baseline \((5 \times 10^{-7} \text{ and } 5 \times 10^{-6}, \text{ respectively})\). This is considered principally because we are not aware of assays that directly measure the motility of inhibitors such as the ones considered in [3]. With low diffusivity, the branching pattern of the cells seems similar to that of the baseline case; however, the rate of cell proliferation does not decrease in the 30 h time frame. The time for vessels to reach the tumor is also comparable to the baseline case. On the other hand, high inhibitor diffusivity spreads the inhibitor concentration largely throughout the domain causing no branching at all. In this case, none of the cells ever reach the tumor and the number of vessels (see Fig. 8(d)) remains practically constant.

Recall that the \(k_{on}\) coefficient represents the interaction between the TAFs and the inhibitor, where a small \(k_{on}\) value implies little degradation of the inhibitor by the tumor, and vice versa. When \(k_{on}\) is lowered fivefold from the baseline case, the cells proliferate at a slower rate than the baseline, and the cells nearest the inhibitor first reach the tumor between 22 to 25 h. Conversely, the large \(k_{on}\) value increases the rate of cell growth, and the point at which cell growth slows down is later than that of the baseline case. With the larger degradation, all the endothelial cells in the vicinity of the inhibitor reach the tumor for typical time scales of approximately 17 h.

Increasing the potency of the inhibitor also reduces the rate of branching, as well as the cells’ paths to the tumor. The cells near the inhibitor take between 19.5 and 22 h to reach the tumor. It should be noted here that Fig. 8(g) seems to counter-intuitively indicate that the number of cells is rather large despite the increased inhibitor potency. This feature is due to the fact that the cells take longer to arrive at the tumor, and hence have a more significant potential for branching (which, in turn, leads to a comparable number of vessels with the previous examples).

4. Conclusions and future challenges

In this paper, we have examined the effect of inhibitor inclusion into a model for the cornea based on the earlier work of [14]. We have observed the effect of changing the geometry, diffusivity, and concentration of the inhibitor. Provided that a replenishment of the inhibitor occurs at sufficiently frequent intervals of time, results obtained include directed cell motion to the tumor (for weak or weakly diffusing inhibitors), tortuous and geometric paths circumventing the inhibitor as well as responses where angiogenesis fails for strong enough concentrations and diffusivities of the inhibitor. These results are qualitatively consistent with the observations of [3] which suggests there is a strong angiogenic response in the absence of an inhibitor; however, when an inhibitor is present, the angiogenic response is prevented and cell vascular structures do not reach the pellet. This is similar to what is observed, e.g. in Fig. 3B of [3].

These results suggest that a closer examination of the role of the inhibitors, such as thrombospondin, could be quite relevant in the prevention of tumor-induced angiogenesis. In this paper, we are not distinguishing between artificial and natural inhibitors even though the model is, in principle, capable of that (such inhibitors would presumably have different physical properties, leading to different mathematical parameters). The possibility of multiple inhibitors is another very interesting direction for future work. A glimpse of the latter can perhaps be inferred from our circumscribed inhibitor setting (see e.g., Fig. 3). The latter is indicative of a setting where multiple (in practice four in this setting) inhibitors are present; in that case, the cells form capillary sprouts permeating the regions between the inhibitors. However, this is a topic meriting separate additional investigation, also as a function of the inhibitors’ strength.

Our results also indicate that appropriate inhibitor replenishment strategies should also be included in order to potentially develop protocols for regulating and potentially avoiding the vascularization of the tumor. Creating a three-dimensional model is another consideration for future work in order to develop a more realistic and practical tumor-induced angiogenesis model in the presence of an inhibitor; such work is currently in progress. Another direction of interest is that of trying to examine quantitatively the results of inhibition in in-vitro experimental settings such as the one of [12]; such an effort to benchmark mathematical/theoretical models appears to be of paramount importance in considerably expanding the impact of mathematical modelling of angiogenesis and related processes.
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