Two-dimensional models of tumour angiogenesis and anti-angiogenesis strategies

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There is a very strong link between the vascularization of a tumour and the spread of the disease, both locally and to distant sites (Gimbrone et al., 1974, J. Natl. Cancer Inst. 52, 413-27; Muthukkaruppan et al., 1982, J. Natl. Cancer Inst. 69, 699-704; Ellis & Fidler, 1995, Lancet 346, 388-9). A tumour becomes vascularized by a process known as angiogenesis. Tumour angiogenesis is initiated by the release of diffusible substances by the tumour, whereby neighbouring capillary vessels are stimulated to grow and eventually penetrate the tumour. Anti-angiogenesis has been proposed as a potential strategy for the treatment of cancer (Folkman, 1995, Nature Med. 1, 21-31; Harris et al., 1996, Breast Cancer Res. Treat. 38, 97-108). In this paper, a mathematical model of the development of the tumour vasculature is presented. By suitable manipulation of the model parameters, we simulate various anti-angiogenesis strategies and we examine the roles that haptotaxis and chemotaxis may play during the growth of the neovasculature. The model is simulated in two space dimensions (on a square domain) so that it is, in theory, experimentally reproducible and any predictions of the model can therefore be tested.

Keywords: tumour angiogenesis; chemotaxis; haptotaxis; anti-angiogenesis.

1. Introduction

The most common types of cancer are carcinomas (cancers of epithelia), which are solid tumours of the internal and external surfaces of tissues and organs. In the majority of patients suffering from carcinomas, the disease has already spread (metastasized) before detection, resulting in multiple secondary tumours (metastases), which may occur in sites far removed from the primary cancer. Hence the disease cannot be cured by treating the primary tumour alone. Widespread metastases can be difficult to treat, and they often prove fatal. Thus, it is highly desirable to prevent metastasis from occurring and so it is important to investigate the mechanisms by which the cancer spreads before the secondary tumours have developed.

The primary tumour is often vascularized; that is, it has its own blood supply and microcirculation. A vascularized tumour has two distinct advantages over an avascular tumour:

(i) the direct supply of nutrients into the tumour results in a rapid increase in growth;
(ii) the tumour can shed cells into the bloodstream, which may consequently lead to metastases.

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A tumour becomes vascularized by a process called angiogenesis. Initially the tumour is avascular; that is, it does not have its own blood supply. The tumour is very small at this stage (1–3 mm in diameter), and it takes in nutrients and expels waste products by diffusion mechanisms alone. However, this mechanism limits the growth of the tumour (Edelstein-Keshet, 1988; LaBarbera & Vogel, 1982).

As the tumour grows, demand increases and nutrients diffusing through the surface of the tumour are used up before they can reach the centre. Cells at the middle of the tumour are starved of nutrients and begin to die (Folkman, 1985). A necrotic core will develop, and eventually there will be an equilibrium between the necrotic cells at the centre and the outer layer of proliferating cells. The tumour will become dormant and growth will stop.

The tumour can overcome this deficiency by acquiring a blood supply, and it does so by inducing any neighbouring blood vessels to grow towards the tumour (Gimbrone et al., 1974; Muthukkaruppan et al., 1982). The formation of these blood vessels is called angiogenesis. Angiogenesis is not unique to tumour growth; it is evident in many other pathological conditions such as diabetic retinopathy, arthritis, and chronic inflammation (Maragoudakis et al., 1992; Paweletz & Knierim, 1989). Tumour angiogenesis is initiated by the release of certain chemicals, known as tumour angiogenesis factors (TAFs), by the tumour (Folkman & Klagsbrun, 1987). This stimulates the endothelial cells (ECs) in neighbouring blood vessels to migrate towards the tumour. Capillary sprouts are formed which grow in length by recruiting ECs from the parent vessel (Ausprunk & Folkman, 1977). Neighbouring sprouts will eventually fuse together at their tips to form loops (anastomoses). The looped vessels themselves may bud or may fuse with other loops until a complex network of vessels develop. This network will eventually penetrate the tumour and furnish it with the nutrients it requires for continued growth.

In this paper, we will develop a two-dimensional model of capillary-vessel formation. As in a previous, one-dimensional model of angiogenesis by Orme & Chaplain (1996), we examine the role that haptotaxis may play during the growth of the neovascularature. It seems clear that anti-angiogenesis strategies could be used to augment existing treatment modalities (Folkman, 1985; Harris et al., 1996). Mathematical models such as the one given here can help us to understand the mechanisms behind angiogenesis and to identify the different ways by which the angiogenic process can be interrupted. By suitable manipulation of the model parameters, we simulate a variety of anti-angiogenic strategies and examine the effect on the pattern of capillary growth. Finally, we suggest an alternative mechanism to capillary-vessel formation which does not involve haptotaxis.

2. Mathematical model

This mathematical model is based on the assumption that diffusible chemicals, generically referred to as TAFs, are released by the tumour cells and that these chemicals stimulate the ECs in nearby blood vessels to grow and migrate towards the tumour. It is known that different TAFs provoke different responses in ECs (Folkman & Klagsbrun, 1987). For example:

(i) some TAFs act as a chemoattractant, whereby the ECs move up the chemical gradient towards the tumour (chemotaxis);
(ii) some TAFs induce the ECs into secreting adhesive substances (for example, fibronectin and collagens) and this creates an adhesive gradient which the ECs move up (haptotaxis, Carter, 1965).

If \( n(x,t) \) denotes the density of the ECs, \( c(x,t) \) the concentration of TAFs, \( p(x,t) \) the density of an adhesive chemical, such as fibronectin, at position \( x \) and time \( t \), then the general conservation equations are

\[
\frac{\partial n}{\partial t} = - \nabla \cdot J + P_1(n, p, c),
\]

\[
\frac{\partial p}{\partial t} = D_2 \nabla^2 p + S_1(n, p, c) + P_2(n, p, c),
\]

\[
\frac{\partial c}{\partial t} = D_3 \nabla^2 c + S_2(n, p, c) + P_3(n, p, c),
\]

where \( J \) is the flux of the ECs, the \( D_i \) (\( i = 2, 3 \)) are the (constant) diffusion coefficients, the \( P_i \) (\( i = 1, 2, 3 \)) are net production/loss terms, and the \( S_i \) (\( i = 1, 2 \)) are sink terms modelling the uptake of the chemotactic/haptotactic chemical by the ECs. These terms will be made explicit below.

First, we assume that the flux of the ECs is governed by diffusion, haptotactic and chemotactic movement, so that we have

\[
J = -D_1 \nabla n + \chi n \nabla p + \kappa n \nabla c,
\]

where \( D_1 \) is the diffusion coefficient, \( \chi \) is the haptotaxis coefficient and \( \kappa \) is the chemotaxis coefficient. For simplicity, we assume that \( D_1, \chi, \) and \( \kappa \) are constant. We further assume that the proliferation of the ECs is governed by logistic-type growth and that any cell loss is linear. Hence we assume that \( P_1 \) takes the form

\[
P_1(n, p, c) = \mu n \left( 1 - \frac{n}{n_0} \right) - \beta n,
\]

where \( \mu \) is the proliferation rate of the cells, \( n_0 \) is the maximum sustainable cell density, and \( \beta \) is the rate of cell loss. In their one-dimensional model of tumour angiogenesis, Chaplain et al. (1995) considered the first-order loss term \(-\beta n\) to be a loss due to the formation of secondary capillary buds. In two or more dimensions, however, such a term represents a loss due to cell death. Since ECs have a long half life (Paweletz & Knierim, 1989) we can assume that any death is due to external intervention, such as the introduction of a cytotoxic drug. In this model, we suppose that the initial release of TAFs induces the ECs into secreting an adhesive (haptotactic) chemical \( p \), which saturates as \( p \) increases. If \( B \) is the threshold level of the haptotactic chemical—above which the production of \( p \) by the ECs is switched on—and if \( \alpha \) is the maximum production rate per cell, then

\[
P_2 = \frac{\alpha n p}{B + p} - \text{decay}.
\]

Here, the chemical-production term shows the response of the ECs to changes in the adhesive chemical density, and this term saturates as \( p \) increases, as required. We assume that any uptake of the haptotactic and chemotactic chemical by the ECs is of the form

\[
S_1 = -s_1 np, \quad S_2 = -s_2 nc,
\]
where \( s_1 \) and \( s_2 \) are the rates of uptake of the haptotactic and chemotactic chemical per cell. The decay of the two chemicals is assumed to be linear. Hence, the full model is

\[
\frac{\partial n}{\partial t} = D_1 \nabla^2 n - \chi \nabla \cdot (n \nabla p) - \kappa \nabla \cdot (n \nabla c) + \mu n \left( 1 - \frac{n}{n_0} \right) - \beta n, \tag{2.4}
\]

\[
\frac{\partial p}{\partial t} = D_2 \nabla^2 p + \frac{anp}{B + p} - s_1 np - \lambda_1 p, \tag{2.5}
\]

\[
\frac{\partial c}{\partial t} = D_3 \nabla^2 c - s_2 nc - \lambda_2 c. \tag{2.6}
\]

We assume a two-dimensional geometry in which the model equations hold on the square domain \( D = [0, L] \times [0, L] \). We assume that the tumour is located along the \( x \)-axis and that the parent capillary vessel lies along the line \( y = L \) so that \( L \) is the perpendicular distance from the tumour to the parent vessel. By using this two-dimensional geometry, the model is, in theory, experimentally reproducible. For example, this model could represent an in vitro experiment, whereby tumour cells are placed in a line along one edge of a square Petri dish with ECs placed along the opposite edge. Alternatively, we could focus upon haptotaxis by suspending TAFs in a gel, so that the gradient of TAFs is constant.

In order to normalize the equations, we define the following reference variables. Let \( n_0 \) be a reference EC density, such as the carrying capacity of the system, let \( p_0 \) be a typical density of the adhesive chemical during angiogenesis, and let \( c_0 \) be the initial density of TAF concentration at the tumour boundary. Hence, nondimensionalize by making the following substitutions:

\[
\begin{align*}
\tilde{n} &= \frac{n}{n_0}, & \tilde{p} &= \frac{p}{p_0}, & \tilde{c} &= \frac{c}{c_0}, & \tilde{t} &= \frac{t}{\tau}, & \tilde{x} &= \frac{x}{L}, & \tilde{y} &= \frac{y}{L}, \\
\tilde{x} &= \frac{p_0 \tau x}{L^2}, & \tilde{x} &= \frac{c_0 \tau \kappa}{L^2}, & \tilde{\mu} &= \mu \tau, & \tilde{\beta} &= \beta \tau, & \tilde{D_i} &= \frac{D_i \tau}{L^2} \quad (i = 1, 2, 3), \\
\tilde{\alpha} &= \frac{an_0 \tau}{p_0}, & \tau &= \frac{1}{s_1 n_0}, & \tilde{s_2} - \tilde{s_1} &= \frac{B}{p_0}, & \tilde{\lambda_1} &= \lambda_1 \tau \quad (i = 1, 2). \tag{2.7}
\end{align*}
\]

Dropping the tildes for notational convenience, the full model equations are

\[
\frac{\partial n}{\partial t} = D_1 \left( \frac{\partial^2 n}{\partial x^2} + \frac{\partial^2 n}{\partial y^2} \right) - \chi \left[ \frac{\partial p}{\partial x} + \frac{\partial n}{\partial x} \frac{\partial p}{\partial y} + n \left( \frac{\partial^2 p}{\partial x^2} + \frac{\partial^2 p}{\partial y^2} \right) \right] - \kappa \left[ \frac{\partial n}{\partial x} \frac{\partial c}{\partial x} + \frac{\partial n}{\partial y} \frac{\partial c}{\partial y} + n \left( \frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2} \right) \right] + \mu n (1 - n) - \beta n, \tag{2.8}
\]

\[
\frac{\partial p}{\partial t} = D_2 \left( \frac{\partial^2 p}{\partial x^2} + \frac{\partial^2 p}{\partial y^2} \right) + \frac{anp}{B + p} - np - \lambda_1 p, \tag{2.9}
\]

\[
\frac{\partial c}{\partial t} = D_3 \left( \frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2} \right) - s_2 nc - \lambda_2 c. \tag{2.10}
\]
2.1 The initial and boundary conditions

The initial conditions are:

(i) if \( y \geq 0.9 \) and \( 0.11 \leq x \leq 0.17 \) and \( 0.35 \leq x \leq 0.41 \) and \( 0.59 \leq x \leq 0.65 \) and \( 0.83 \leq x \leq 0.89 \), then \( n(x, y, 0) = 1 \), otherwise \( n(x, y, 0) = 0 \);
(ii) \( p(x, y, 0) = \frac{1}{2} n(x, y, 0) \);
(iii) \( c(x, y, 0) = 1 - y \).

Thus, we assume that there are initially four capillary sprouts and, equivalently, four foci of fibronectin. For all the numerical simulations, we took zero-flux boundary conditions for \( n \) and \( p \), except for the ECs at the boundary \( y = 1 \). At this boundary, the four capillary sprouts remain in contact with the parent vessel, allowing continual recruitment of ECs from the parent vessel through the degraded basement membrane, and hence we impose the boundary condition: if \( 0.11 \leq x \leq 0.17 \) and \( 0.35 \leq x \leq 0.41 \) and \( 0.59 \leq x \leq 0.65 \) and \( 0.83 \leq x \leq 0.89 \), then \( n(x, y = 1, t) = 1 \). From the experimental results, it is evident that, apart from recruitment from the parental vessel via these (in this case, four) initial capillary sprouts, the ECs do not cross the basement membrane of the parent vessel anywhere else (Pawel et al., 1989). We thus impose zero-flux boundary conditions on \( n \) throughout the remaining part of the boundary \( y = 1 \). The appropriate boundary conditions for \( c \) are \( c(x, 0, t) = 1 \), \( c(x, 1, t) = 0 \), \( c_x(0, y, t) = c_x(1, y, t) = 0 \), which reflect the fact that we assume the TAF concentration is held constant at some fixed level at the site of the tumour (\( y = 0 \)), that it has decayed to zero at the parental vessel (\( y = 1 \)), and that it must remain within the Petri dish. In Section 2.2, we simplify the model by assuming that the TAF has reached a steady state. This is analogous to an experiment where a Petri dish contains TAF suspended in a gel, say.

2.2 A model simplification

The full model (2.8–2.10) is time consuming to solve numerically and it has a large parameter set, which makes analysis of the numerical results more difficult from a biological viewpoint. To make the problem tractable, we can simplify system (2.8–2.10) in various ways, in order to emphasize some key aspect of tumour angiogenesis. In this paper, we place a particular emphasis on haptotaxis.

In order to focus attention upon the role of haptotaxis in angiogenesis, we simplify the profile of the chemotactic chemical. The TAF concentration profile studied by Chaplain et al. (1995) did not vary drastically over time. They concluded that it is reasonable to assume that the TAF profile is in some kind of steady state, since the TAF diffuses much faster than the ECs. In the numerical simulations presented in Section 3, we assume that the TAF has reached its steady state and we approximate the TAF profile by \( c(x, y) = 1 - y \) (cf. Chaplain et al., 1995). Thus, in Section 3, we solve numerically the simplified model,

\[
\frac{\partial n}{\partial t} = D_1 \left( \frac{\partial^2 n}{\partial x^2} + \frac{\partial^2 n}{\partial y^2} \right) - \chi \left[ \frac{\partial n}{\partial x} \frac{\partial p}{\partial x} + \frac{\partial n}{\partial y} \frac{\partial p}{\partial y} + n \left( \frac{\partial^2 p}{\partial x^2} + \frac{\partial^2 p}{\partial y^2} \right) \right] + \kappa \frac{\partial n}{\partial y} + \mu n (1 - n) - \beta n,
\]

(2.11)
\[ \frac{\partial p}{\partial t} = D_2 \left( \frac{\partial^2 p}{\partial x^2} + \frac{\partial^2 p}{\partial y^2} \right) + \frac{\alpha n p}{B + p} - np - \lambda_1 p. \] (2.12)

2.3 Estimation of parameter values

Whenever possible, experimental data was used to estimate the parameter values. If this was not possible, then the parameters were chosen in order to give the best qualitative results.

2.3.1 Estimation of fibronectin uptake time \( \tau \). Terranova et al. (1985) found that doses of fibronectin between \( 10^{-8} \) M and \( 10^{-10} \) M stimulated cell migration, and Yamada & Olden (1978) gave the generation time of fibronectin to be 18 h. Assuming that \( 10^{-8} \) M of fibronectin is produced by \( n_0 \) cells in 18 h, we have estimated the rate of the secretion of the fibronectin as \( (10^{-8}/18)n_0h^{-1} \) M (Orme & Chaplain, 1996). We would expect the secretion rate to be higher than the uptake rate, say five times higher; that is, \( \alpha = 5 \). Hence taking \( p_0 \) in the range \( 10^{-8} - 10^{-10} \) M (Terranova et al., 1985), we have \( \tau = 1/s_1n_0 \) in the range 90–9000 h.

2.3.2 Estimation of the diffusion coefficients \( D_1 \) and \( D_2 \). In their model of epidermal wound healing, Sherratt & Murray (1990) used values of \( 3 \times 10^{-9} \) cm\(^2\) s\(^{-1}\), \( 3.5 \times 10^{-8} \) cm\(^2\) s\(^{-1}\), and \( 6.9 \times 10^{-11} \) cm\(^2\) s\(^{-1}\) for the diffusion of the cells. In their study of individual ECs, Stokes et al. (1991) calculated a random motility coefficient of (7.1 ± 2.7) \( \times 10^{-9} \) cm\(^2\) s\(^{-1}\) for ECs migrating in a culture containing an angiogenic factor \( \alpha \)FGF (Folkman & Klagsbrun, 1987), heparin, and fetal calf serum. Assuming that the diffusion of the ECs is in the range \( 10^{-9} - 10^{-11} \) cm\(^2\) s\(^{-1}\), then \( D_1 \) is in the range \( 3.6 \times 10^{-5} - 5.1 \). Since we want to focus upon the roles of haptotaxis and chemotaxis in this model, we want the diffusion coefficient to be as small as possible without running into difficulties with the numerical simulations. Hence, we chose \( D_1 = 0.0025 \).

For the diffusion coefficient of the chemotactic chemical, Sherratt & Murray (1990) took values of \( 3.1 \times 10^{-7} \) cm\(^2\) s\(^{-1}\) and \( 5.9 \times 10^{-6} \) cm\(^2\) s\(^{-1}\) and Chaplain et al. (1995) took \( 3.3 \times 10^{-8} \) cm\(^2\) s\(^{-1}\). Assuming that the diffusion coefficient of the haptotactic chemical is in the range \( 10^{-6} - 10^{-8} \) cm\(^2\) s\(^{-1}\), we obtain \( D_2 \) in the range \( 3.6 \times 10^{-2} - 5.1 \). We chose \( D_2 = 0.5 \), which is in the middle of the range and is 200 times larger than the diffusion coefficient of the tumour cells.

2.3.3 The haptotaxis coefficient \( \chi \) and chemotaxis coefficient \( \kappa \). In the absence of reliable empirical data, we chose \( \chi = 0.5 \), which leads to a dimensional value of the haptotaxis coefficient in the range \( 10^{-2} - 1400 \) cm\(^2\) s\(^{-1}\) M\(^{-1}\). Stokes et al. (1991) estimated the chemotaxis coefficient of ECs migrating in a culture containing \( \alpha \)FGF, to be \( 2600 \) cm\(^2\) s\(^{-1}\) M\(^{-1}\). Choosing \( \kappa = 0.65 \), the nondimensionalization gives a value of \( c_0 \) in the range \( 6.7 \times 10^{-11} - 4.9 \times 10^{-14} \) M. From this, we can infer that the TAF mobilizes ECs at a smaller concentration than that of the fibronectin.
2.3.4 **The proliferation rate $\mu$ and the death rate $\beta$.** The proliferation rate of the ECs is estimated to be in the range $0.04$ h$^{-1}$ (Sherratt & Murray, 1990) to $0.056$ h$^{-1}$ (Stokes & Lauffenburger, 1991), assuming that all the cells undergo mitosis. However, fibronectin can inhibit EC proliferation by up to 23% (Bowersox & Sorgente, 1982). Furthermore, during angiogenesis, proliferation is generally confined to a region near the tips of the capillary sprouts. Hence, by assuming that the proliferation rate is $0.02$ h$^{-1}$ (Chaplain *et al.*, 1995; Stokes & Lauffenburger, 1991), we obtain $\mu$ in the range $1.8$–$180$.

Brooks *et al* (1994) found that ECs underwent apoptosis $48$ h after injecting integrin $\alpha_v\beta_3$ antagonists into the site. Taking $1/48$ h$^{-1}$ as the death rate, we have $\beta$ in the range $1.9$–$190$.

2.3.5 **The fibronectin density ratio $B$.** We would expect that the secretion of fibronectin is switched on by the initial release of TAF by the tumour, so that the typical threshold fibronectin density $p_0$ is greater than the threshold level; that is $B < 1$. We take $B = 0.001$.

2.3.6 **Decay of haptotactic and chemotactic chemicals $\lambda_1$.** If we take $\lambda_1 = 0.5$, then the dimensional estimate for the decay of the chemicals is $5.6 \times 10^{-3} - 5.6 \times 10^{-5}$ h$^{-1}$.
3. Numerical simulations of the simplified model

The system of equations (2.11–2.12) was solved using a routine available from the National Algorithms Group (NAG) library, which performs integrations using the method of lines and Gear's method. In our first numerical simulation, we solved the system (2.11–2.12) with parameters \( \kappa = 0.65, \mu = 5, \beta = 0, \chi = 0.5, \lambda_1 = 0.5, \alpha = 5, B = 0.001, D_1 = 0.0025, \) and \( D_2 = 0.5. \) Figures 1 and 2 show the resultant growth of the capillary vessels through the host tissue. In Fig. 1, we can see the beginning of secondary branch formation at the tip of each capillary sprout, and these branches subsequently merge to form anastomoses (Fig. 2) via branch-tip-to-branch-tip fusion (Konerding et al., 1992). Furthermore, the capillaries have a well-defined structure (cf. Fig. 8), which is necessary for blood to flow through the vessels. Note that there is a higher density of tumour cells at the front of the capillary vessel; this is the brush-border effect observed by Muthukkaruppan et al. (1982). The distribution of the haptotactic chemical after a time \( t = 1.2 \) is shown in Fig. 3.

3.1 Anti-angiogenesis strategies

In the numerical simulations, we consider four different ways by which the angiogenic process can be disrupted. These four approaches represent viable anti-angiogenesis strategies,
which can be used in conjunction with more established treatment modalities (Folkman, 1995; Norton, 1995). Furthermore, we explicitly target the endothelium in the neovascularure so that, in theory, the damage to normal tissue is minimized (Brooks et al., 1994; Folkman, 1995).

3.1.1 Cytotoxic targeting of ECs. First, we consider the use of cytotoxic therapy which preferentially kills ECs. Such a strategy is most beneficial if pre-existing blood vessels can be left unaffected. For example, Brooks et al. (1994) demonstrated that antagonists of integrin $\alpha_v\beta_3$ disrupted tumour angiogenesis by selectively inducing apoptosis in ECs during the proliferative phase of the cell cycle. Since only the ECs in the neovascularure undergo mitosis on the time scale under consideration, the adjacent vessels are left intact. We model this by setting $\beta = 50$. As expected, such a strategy results in the complete regression of the capillary sprouts (see Fig. 4).

3.1.2 Inhibition of cell mitosis. It has been demonstrated that proliferation of ECs is vital for the successful completion of angiogenesis (Ausprunk & Folkman, 1977; Paweletz & Knierim, 1989). Recently, chemical agents, such as angiostatin (Folkman, 1995; O’Reilly et al., 1994) have been isolated which specifically inhibit EC proliferation and thus inhibit angiogenesis. Since the cell-doubling time of ECs in the absence of TAF is long,
months (Paweletz & Knierim, 1989), in comparison with the half-life of angiotatin, 2.5 days (Folkman, 1995), only the newly formed vasculature would be affected. We model this by setting the cell-proliferation rate $\mu$ to zero. In the numerical simulation, the capillary sprouts stopped growing after a time $t = 0.1$ (Fig. 5). O'Reilly et al. (1994) found that angiotatin inhibited angiogenesis 48 h after implantation, which gives us a value of $\tau = 480$, which is within our estimated range.

3.1.3 Prevention of cell migration: (1) Anti-chemotaxis. Cell migration has been identified as a key event in tumour angiogenesis. Cell migration can be disrupted by interfering with the cells' ability to detect local chemical gradients. For example, ECs are known to react chemotactically to hepatocyte growth factor (HGF) (Bussolino et al., 1992). It is possible to cultivate antibodies against the HGF receptor (Bussolino et al., 1992), and hence prevent chemotaxis. We modelled this by setting our chemotaxis coefficient $\kappa$ to zero to obtain the numerical solution shown in Fig. 6. This shows that in the absence of a detectable TAF gradient angiogenesis fails.

3.1.4 Prevention of cell migration: (2) Anti-haptotaxis. It is known that fibronectin increases cell-cell and cell-matrix adhesiveness. Yamada & Olden (1978) showed that ECs
have a specific receptor for fibronectin. By blocking the fibronectin receptors, we prevent the ECs from reacting haptotactically to fibronectin. We modelled this by setting the haptotaxis coefficient $\chi$ to zero. At first, this method does not seem to have impeded the growth of the capillary sprouts. At time $t = 0.6$ there is evidence of anastomoses (Fig. 7), though the loops do not appear to have been formed by the fusion of branch tips. Furthermore, the capillary vessels are not as distinct as those in Figs. 1 and 2. This is more evident in Fig. 8 where we can see that the ECs have not formed well-defined structures. We would expect the circulation of blood through such inferior vessels to be poor, and hence the angiogenic process has failed to produce a viable network of capillaries. Hence, we conclude that the greater compactness of the vessels shown in Figs. 1 and 2 than those in Figs. 7 and 8 is due to haptotaxis.

Finally, we conducted a parameter-sensitivity analysis on this model and found that the following parameter changes had an equivalent effect on the resultant solutions. Increasing the proliferation rate, increasing the diffusion coefficient of the haptotactic chemical, or decreasing the haptotaxis coefficient all resulted in a loss of definition (compactness) of the capillary sprouts (a similar result is shown in Fig. 8). Furthermore, the same effect could be achieved by increasing or decreasing the secretion rate of the haptotactic chemical. This implies that there is an optimal level of (fibronectin) production, at which the ECs cannot
respond to too little chemical or become saturated when there is too much. A summary of the eight numerical simulations conducted is given in Table 1.

4. An alternative chemotaxis model

We will now briefly consider an alternative mechanism for the formation of capillary sprouts during angiogenesis which does not involve haptotaxis. We assume that the TAF produced by the tumour does not induce the secretion of fibronectin (or any other such adhesive material) by the ECs. Hence, by setting $p(x, y, t) = 0$ in system (2.8–2.10), we can obtain the submodel

$$\frac{\partial n}{\partial t} = D_1 \left( \frac{\partial^2 n}{\partial x^2} + \frac{\partial^2 n}{\partial y^2} \right) - \kappa \left[ \frac{\partial n}{\partial x} \frac{\partial c}{\partial x} + \frac{\partial n}{\partial y} \frac{\partial c}{\partial y} + n \left( \frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2} \right) \right] + \mu n(1 - n) - \beta n,$$

$$\frac{\partial c}{\partial t} = D_3 \left( \frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2} \right) - s_2 nc - \lambda_2 c,$$

(4.1) (4.2)
Fig. 7. A numerical solution of the system (2.11-2.12) plotting the distribution of the ECs, \( n \), at time \( t = 0.6 \) with the fixed TAF profile and the parameter values given in Fig. 1 except that \( \chi = 0 \). The anti-angiogenesis strategy adopted here prevents the ECs from reacting to the haptotactic chemical, for example, by affecting the receptors on the cell surface. Though the capillary sprouts continue to grow under the influence of chemotaxis, the sprouts are not as distinct as those in Figs. 1 and 2.

### Table 1

A summary of the eight different anti-angiogenesis strategies and a parameter-sensitivity analysis and the effect on the solution as compared to Figs. 1 and 2

<table>
<thead>
<tr>
<th>Action</th>
<th>Change in parameter</th>
<th>Effect on solution</th>
</tr>
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<tbody>
<tr>
<td>Cytotoxic targeting of ECs (Brooks* et al., 1994)</td>
<td>( \beta = 50 )</td>
<td>Complete regression of capillary sprouts</td>
</tr>
<tr>
<td>Inhibiting EC mitosis (O’Reilly* et al., 1994)</td>
<td>( \mu = 0 )</td>
<td>Capillary sprouts stop growing</td>
</tr>
<tr>
<td>Anti-chemotaxis</td>
<td>( \kappa = 0 )</td>
<td>Capillary sprouts stop growing</td>
</tr>
<tr>
<td>Anti-haptotaxis</td>
<td>Increase ( \mu )</td>
<td>Loss of compactness</td>
</tr>
<tr>
<td>Increasing proliferation</td>
<td>Increase ( D_2 )</td>
<td>Loss of compactness</td>
</tr>
<tr>
<td>Increasing diffusion of haptotactic chemical</td>
<td>Increase ( \alpha )</td>
<td>Loss of compactness</td>
</tr>
<tr>
<td>Increasing secretion of haptotactic chemical</td>
<td>Decrease ( \alpha )</td>
<td>Loss of compactness</td>
</tr>
<tr>
<td>Decreasing secretion of haptotactic chemical</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discuss haptotaxis and sprouts, especially in the context of ECs.

FIG. 1.2. Tb = ...

Dissection of the role of ECs in angiogenesis.

2.1 A model of EC behavior...

3.1 Another model of EC behavior...

4.1 An alternative model of EC behavior...

5.1 Another alternative model of EC behavior...