A mathematical model of the first steps of tumour-related angiogenesis: Capillary sprout formation and secondary branching

M. E. Orme and M. A. J. Chaplain

School of Mathematical Sciences, University of Bath, Bath BA2 7AY, UK

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The growth of a solid tumour is dependent on an adequate supply of nutrients. A tumour can establish a blood supply by inducing neighbouring blood vessels to sprout and grow towards it, a process known as angiogenesis. The tumour cells may secrete a number of diffusible chemicals which stimulate endothelial cells to migrate, to rearrange themselves into capillary tubes or sprouts, and to proliferate. In this paper we focus firstly upon the early stage of angiogenesis wherein the endothelial cells group together in the parent vessel to form the initial capillary-sprout buds. A mathematical model for the formation of the capillary buds is presented which focuses on the potential role that haptotaxis may play. In Section 2 we turn attention to the endothelial cells within the growing and developing capillary sprouts as they migrate towards the tumour cells. Once again the potential role of haptotaxis is focused upon.

Keywords: angiogenesis; endothelial cells; sprout formation; haptotaxis.

1. Introduction

The growth of most solid tumours takes place in two stages [11]. In the first stage, the tumour is avascular; that is, it does not have a network of capillary vessels supplying it with nutrients. During this avascular stage, the tumour may be considered as being roughly spherical in shape consisting of a central necrotic core surrounded by a layer of quiescent cells which is, in turn, surrounded by a thin layer of proliferating cells [36]. At this stage the tumour is small enough (1–3 mm in diameter) to take in nutrients and expel waste products by diffusion alone. However, diffusion is not sufficient to support any continued growth of the tumour. This is because the tumour consumes nutrients at a rate which is proportional to its volume, whereas the supply of nutrients is delivered at a rate proportional to its surface area. The avascular tumour can sometimes become dormant, and there is then an indefinite period for which growth stops.

The inadequacy of diffusion as a means of transportation of vital nutrients has been demonstrated, for example, by LaBarbera & Vogel [22] and Edelstein-Keshet [9]. By applying Fick’s first law of diffusion across a wall (e.g. of a cell) of thickness \( dx \), the diffusive flux of a nutrient across the surface is given by

\[
J = DS \frac{dC}{dx},
\]

where \( C \) is the concentration of the nutrient, \( D \) is the diffusion coefficient, and \( S \) is
the surface area \([19, 22]\). Suppose then we have a spherical tumour, with radius \(r\), volume \(V = \frac{4}{3}\pi r^3\) and surface area \(S = 4\pi r^2\). Suppose the concentration of a given substance (e.g. oxygen) at the tumour surface is \(c_0\) and the tumour uses the substance completely so that at \(r = 0\), \(c(0, t) = 0\) (cf. [17]), where \(c\) is the concentration of the substance. Crudely speaking, the total diffusive flux across the tumour surface will be

\[
J = DS \frac{c_0}{r} = 4\pi D c_0 r,
\]

where the gradient has been approximated by \(c_0/r\), that is, by the concentration difference per unit distance \([9]\). The rate at which the substance is depleted will be proportional to the tumour's volume. If \(\tau\) is a fixed constant representing the time it takes for the substance to be used up completely then

\[
\textit{rate at which substance used} = \frac{4\pi r^3}{3\tau}.
\]

Hence,

\[
\frac{\text{rate of supply}}{\text{rate used}} \approx \frac{3D c_0 \tau}{r^2}.
\]

In order to meet the demands of the tumour the rate of supply of the substance must be greater or equal to the rate at which the substance is used; that is, the ratio must be greater than 1. Thus a minimum requirement is

\[
c_0 = \frac{r^2}{3D\tau}.
\]

So the external substance concentration must be at the very least proportional to the square of the radius of the tumour. This is unrealistic if the radius of the tumour is large. Hence, as the tumour grows, cells at the centre of the tumour become starved of nutrients and begin to die \([17]\). Dormancy will occur when the necrosis at the centre of the tumour and the proliferation of the outer layer are in equilibrium. The tumour can overcome this deficiency by acquiring a blood supply which can deliver nutrients directly into the tumour.

During the second stage of growth, the tumour becomes vascular; that is, it is penetrated by capillary vessels. A vascularized tumour can grow exponentially, and there is the possibility of both invasion and metastasis \([11]\). Vascularization is realized by a process known as angiogenesis, that is, the formation of blood vessels. Angiogenesis occurs during physiological processes such as embryonic development, or during pathological processes such as wound healing, rheumatoid disease, and of course tumour growth (see the references in \([28]\)).

Various techniques have been developed in order to examine the cascade of events that lead to neovascularization. For example, the cloning of capillary endothelial cells (ECs) in culture is a method used for studying angiogenesis \textit{in vitro} \([12, 18]\). Since \textit{in vitro} studies can only generalize about angiogenesis, \textit{in vivo} studies are also employed in order to explore the specifics of tumour-induced angiogenesis. The corneal micropocket \([25]\) and the hamster-cheek-pouch chamber \([16]\) are just two of the methods used to study angiogenesis \textit{in vivo}. 

The linings of many vessels, such as veins and small lymphatic vessels, are formed from a monolayer of ECs. The ECs lie upon a continuous basement membrane (or basal lamina) and are in close contact with their neighbours. The aforementioned studies have shown that ECs play a crucial role in angiogenesis, and in fact it has been shown, in vitro that ECs can construct capillary networks unaided by other cell types [12].

Angiogenesis is initiated by the release of angiogenic factors from the tumour, though it is not known what triggers this activity. There are two types of angiogenic factors, those that act directly on ECs and those that induce other cells into producing factors which act on ECs [12, 13]. Angiogenic factors induce one or more of the following activities in ECs:

(i) the secretion of proteases and collagenases by ECs which degrade the basal lamina and the extracellular matrix (ECM);
(ii) the migration of ECs towards a chemotactic stimulus; and
(iii) proliferation of ECs.

For successful tumour vascularization, all three events must be carefully orchestrated. In recent years several angiogenic factors have been identified. A summary of these factors has been given by Folkman & Klagsbrun [13]. The suggestion is that several angiogenic factors act together, either directly or indirectly, to promote angiogenesis. We now describe these activities in more detail.

The tumour releases angiogenic factors (TAFs) which diffuse into the surrounding tissue. The first reaction to this stimulus is that the ECs in the neighbouring vessels and those nearest to the chemical source start to alter their structure. The cells thicken, and finger-like protrusions can be observed on the abluminal surface [1, 28]. Cell-associated proteases degrade the basement membrane so that the ECs loosen their contacts with their neighbours. Stimulated by the TAFs the ECs begin to migrate [40]. The ECs accumulate in the region where the concentration of TAFs first reaches a threshold level [28]. The vessel wall begins to bulge as ECs pile up to form sprouts. The gaps in the basement membrane are not large enough to allow ECs into the extracellular matrix beyond the vessel wall. Therefore the ECs secrete proteases and collagenases which dissolve the basal lamina and the surrounding extracellular matrix, enabling the capillary sprout to grow towards the tumour [20].

The capillary sprouts begin to grow in length by recruiting ECs from the parent vessel. At some distance from the tip of the sprout ECs begin to proliferate. There is evidence that migration and mitosis are independent events and that separate TAFs are required to stimulate these activities of ECs [10, 28, 40]. The first stages of angiogenesis can be performed without cell proliferation [33], with mitosis occurring after the capillary sprouts have started to grow. However, proliferation is essential for the completion of angiogenesis since gaps will develop in the parent vessel, resulting in an abnormal permeability [1].

The ECs in the outgrowing sprouts start to reassociate with each other. The development of intracellular lumen (where vacuoles appear within the ECs) and intercellular lumen (where vacuoles appear between the outgrowing sprouts) leads to the formation of tube-like structures [21]. Branches form in a similar way, whereby
an intracellular vacuole becomes Y- or T-shaped before fusing with the primary lumen [12]. Initially the sprouts are parallel with each other, but they tend to move towards each other as they elongate. Neighbouring sprouts will eventually fuse together at their tips to form loops (anastomoses). This signals the beginning of the circulation of blood. It is essential that there is a flow of blood through the tumour since an effective system for transporting waste products away from the tumour is vital. As the vessels mature, the ECs resynthesize a basal lamina, in order to restore continuity. The looped vessels may bud, or loops may fuse with other loops, until a complex network of vessels develop. Finally, this network penetrates the tumour, providing it with the circulatory system and the supply of nutrients that it requires for growth. In order to support continued growth, the tumour’s vascular system persistently remodels itself. Hence angiogenesis is an on-going process, continuing indefinitely until the tumour is removed, or killed, or until the host dies.

Initially the ECs are uniformly distributed along the walls of the parent vessel. The release of diffusible substances from the tumour triggers an angiogenic response in the ECs. The ECs become mobile and form clusters of cells which are the beginning of the buds. These buds will eventually sprout and will form the primary capillary vessels in the tumour’s vascular system. There is clear experimental evidence that the mechanism by which the cells move involves haptotaxis; that is, the cells move up an adhesive gradient, especially in the early stages. Studies have shown that compounds such as fibronectin are secreted by the ECs during the angiogenic process [6, 28], and that fibronectin increases cell-to-cell and cell-to-matrix adhesiveness [7, 39]. It can be supposed that fibronectin aids angiogenesis by directing migration [6, 7, 28, 38]. Chemotaxis is also certainly involved in angiogenesis [5], but we will focus our attention on the potential role of haptotaxis.

The TAF which diffuses from the tumour creates a chemical gradient. In this model, we assume that the ECs secrete fibronectin in response to the TAF and hence move up an adhesive gradient of their own creation. Carter has suggested [4] that a chemotactic substance may act indirectly by altering the surface of a cell, and so increasing its adhesiveness. Fibronectin can bind to cell membranes by means of a specific fibronectin receptor, thereby anchoring the cell to the ECM [29]. An adhesive gradient will be created since the surface which is nearest the chemical stimulus will be altered.

In the rest of this section we develop a mathematical model for the initial formation of capillary sprouts, that is, for the budding process mentioned previously, and we present the results of numerical simulations. In Section 2, the modelling of the branching process is considered and numerical simulations are again presented. The final section is a conclusion.

1.1 Mathematical model

As stated in the previous section, we will initially focus on the early stages, and consider the ECs within the parent vessel, for example, the limbus. Let \( n(x,t) \) be the EC density and let \( c(x,t) \) be the concentration of fibronectin. We assume that there are only two contributions to the cell flux at this early stage of the angiogenic
process—random motion and haptotaxis. Thus
\[ J_n = J_{\text{diffusion}} + J_{\text{haptotaxis}}, \]
where in the usual way \( J_{\text{diffusion}} = -d_n(n)\nabla n \) and \( J_{\text{haptotaxis}} = a(c)n\nabla c \), and where \( d_n \) and \( a \) are the the diffusion and haptotaxis coefficients, respectively. For simplicity we will assume that these variables are constant. If \( N \) is the total number of cells and \( rN \) is the linear mitotic growth rate, then the net cell production can be modelled using a logistic-type growth rate given by \( rN(N - n) \).

We assume that the fibronectin is simply secreted by the ECs and that it diffuses into the surrounding area. The flux of the chemical fibronectin is given by \( J_c = -d_c\nabla c \), where \( d_c \) is the diffusion coefficient of the chemical, which is again assumed to be constant. It is assumed that the production of fibronectin by the cells is given by the Michaelis–Menten production term \( Sn/(\beta + n) \), and that the decay term is \(-\gamma c \), where \( S, \beta, \) and \( \gamma \) are positive constants.

Angiogenic activity is confined to a small region of the parent-vessel wall nearest the tumour [28]. Initially, the vessel wall is simply a monolayer of ECs. After the release of the TAF, some of the ECs loosen their contacts with their neighbours and try to penetrate the basement membrane. Subsequently, several ECs follow the primary ECs and pile up behind each other, creating a bulge in the wall of the capillary. Hence we need only consider the model in one spatial dimension, in order to focus attention on clusters of cells rather than on the overall shape of the buds. We assume that a region of increased cell density indicates the initiation of a cell cluster leading to a bud.

Conservation of mass gives us
\[ n_t + \nabla \cdot J_n = f(n, c), \]
\[ c_t + \nabla \cdot J_c = g(n, c), \]
where \( f \) and \( g \) contain the appropriate source and sink terms as detailed above. Hence the system of equations in one spatial dimension is
\[ n_t = d_n n_{xx} - a(nc_x)_x + rn(N - n), \quad (1) \]
\[ c_t = d_c c_{xx} + \frac{Sn}{(\beta + n)} - \gamma c. \quad (2) \]

During angiogenesis, ECs in the parent vessel actively move towards the tumour [11]. Experimental evidence [25] shows that only a finite region of the parent vessel is involved in producing buds, and hence we will take our spatial domain \( D \) to be finite. Given this geometry, it is not unreasonable to treat the system as closed and to impose the zero-flux boundary conditions
\[ d_n n_x - an c_x = 0, \quad c_x = 0 \quad \text{on} \ x \in \partial D; \]
that is
\[ n_x = c_x = 0 \quad \text{on} \ x \in \partial D. \]
We introduce dimensionless variables into the model in the following manner:

\[ x^* = \left( \frac{\gamma}{d_s \beta} \right)^{\frac{1}{2}}, \quad t^* = \frac{\gamma t}{s}, \quad n^* = \frac{n}{\beta}, \quad c^* = \frac{\gamma c}{S}, \]

\[ N^* = \frac{N}{\beta}, \quad d^* = \frac{d_n}{d_c}, \quad a^* = \frac{aS}{\gamma d_c}, \quad r^* = \frac{r\beta}{\gamma}, \]

where \( s \) is a scale factor which refers to the size of the domain [26]. After nondimensionalizing, and dropping the asterisks for notational convenience, the equations become

\[ n_t = d n_{xx} - a(n c_x)_x + sr n(N - n), \]

\[ c_t = c_{xx} + s\left( \frac{n}{n + 1} - c \right), \]

\[ n_x = c_x = 0, \quad x = 0, 1. \]

Note that the above system of equations models the reaction of the ECs to the TAF which is secreted by the tumour cells.

1.2 Linear stability analysis

The following analysis is standard, but we include it here for completeness. We now look for spatially homogeneous steady states of the above system; these are easily seen to be

\[ (n, c) = (0, 0) \quad \text{and} \quad (n, c) = \left( N, \frac{N}{N + 1} \right). \]

The trivial state is not biologically relevant. We thus linearize about the nontrivial steady state in the usual manner by substituting

\[ n = N + u \quad \text{and} \quad c = \frac{N}{N + 1} + v, \]

where \( |u| \) and \( |v| \) are small, into the above equations to obtain the linearized system

\[ u_t = d u_{xx} - a N v_{xx} - sr N u, \]

\[ v_t = v_{xx} + s\left( \frac{u}{(N + 1)^2} - v \right). \]

We look for solutions of the form \( (u, v) \propto \exp(\sigma t + ikx) \); hence we obtain the dispersion relation

\[ \sigma^2 + \sigma[k^2(1 + d) + s + srN] + k^2\left( d(k^2 + s) + srN - \frac{saN}{(N + 1)^2} \right) + s^2rN = 0. \]

This dispersion relation (a quadratic equation) will have two roots, and these will either be real or complex conjugates.
In the absence of any spatial variation the homogeneous steady state must be linearly stable, so that any instability will be diffusion driven. So by putting $k^2 = 0$ in the dispersion relation and solving for $\sigma$, we get

$$\sigma = -rNs, \quad -s \leq 0.$$ 

So the spatially homogeneous steady state is linearly stable. We require $\text{Re}(\sigma(k^2)) > 0$, for some $k$, for instability. When $\sigma = 0$, we have

$$dk^4 + k^2\left(\frac{aNs}{N + 1} - ds + rN\right) + rns^2 = 0,$$

giving

$$k^2 = \frac{aNs - (ds + rN)(N + 1)^2}{2d(N + 1)^2} \pm \frac{1}{2d}\left[\left(ds + rN - aNs\right)^2 - 4drNs^2\right]^{\frac{1}{2}}. \quad (9)$$

Studies have shown that there is no significant increase in the rate of EC mitosis during the first stages of angiogenesis [1]. Mitosis occurs after the first sprouts have formed. It has been postulated that mitosis is a secondary reaction to angiogenesis, resulting from abnormal permeability of the host vessels. Normal ECs have a long half-life, and cell division is rare, occurring only when repair and remodelling of large wounds is essential [28]. It has also been shown that fibronectin even inhibits cell proliferation to some extent [3]. We will consider separately the case where there is no mitosis and where there is mitosis at some background level.

1.2.1 No mitosis ($r = 0$). Consider the case where there is no mitosis (i.e. $r = 0$), then the system is similar to the model examined by Myerscough & Murray [26]. Then from equation (9) we have the two roots

$$k^2 = \begin{cases} 0, \\ \frac{aNs}{d(N + 1)^2} - s = k^2_2. \end{cases}$$

Hence the dispersion relation is tethered to the origin, which is expected when there is no proliferation of cells. By differentiating the dispersion relation with respect to $k^2$ and setting $r = 0$ we obtain

$$\frac{d\sigma}{dk^2} [k^2(1 + d) + s + 2\sigma] + \sigma(1 + d) + 2dk^2 + ds - \frac{saN}{(N + 1)^2} = 0.$$ 

Setting $\sigma = 0$,

$$\frac{d\sigma}{dk^2} = \frac{aNs}{[s + k^2(d + 1)](N + 1)^2} - \frac{ds + 2dk^2}{s + k^2(d + 1)}.$$ 

So, $d\sigma/dk^2 > 0$ at the origin provided $aNs/d(N + 1)^2 - s > 0$; that is, provided $k^2_2 > 0$. The maximum occurs at $d\sigma/dk^2 = 0$; that is, at $k^2_M$, where

$$\sigma_M(1 + d) + 2dk^2_M + ds - \frac{saN}{(N + 1)^2} = 0.$$
But if the maximum value is $\sigma = 0$ then $k_M^2 = 0$ since the dispersion relation is tethered to the origin as shown in Fig. 1. Hence $a_c = d(N^2 + 1)^2/N$ is the critical value of $a$. So provided $a > a_c$ we will have instability.

1.2.2 Mitosis included ($r > 0$). In this model we will include some mitosis, but at a reduced rate in order to account for the effect of fibronectin on the proliferation of ECs [3]. With $r > 0$, the critical value of $k^2$, $k_2^2$, occurs when equation (9) has only one root, that is, when

$$\left(ds + rNs - \frac{aNs}{(N + 1)^2}\right)^2 - 4drNs^2 = 0,$$

so that

$$k_2^2 = \frac{1}{2d} \left(\frac{aNs}{(N + 1)^2} - ds - rNs\right) = s\left(\frac{rN}{d}\right)^{\frac{1}{2}}.$$

So the critical value of $a$, $a_c$, is given by equation (10). A typical dispersion relation is shown in Fig. 2. Again when $a > a_c$ the system will be unstable for sufficiently small $r$. Also as $r \to 0$, $k_2^2 \to 0$ and $a_c \to d(N + 1)^2/N$, which agrees with our earlier observations.

1.3 Estimation of parameters

Wherever possible, experimental data was used to estimate the parameter values $d$, $a$, $s$, $r$, and $N$. For simplicity we took $S$, $N = 1$. 

FIG 1. A typical dispersion relation where $r = 0$ and $a > a_c$ so that there is instability for $0 < k^2 < k_2^2$. 

FIG 2.
(a) **Michaelis-Menten parameter** $\beta$. We assume that the secretion of fibronectin by the ECs is governed by Michaelis-Menten kinetics. Fibronectin molecules inside the ECs form a complex by combining with receptors on the cell membrane. They are then carried across the membrane and deposited outside the cell. Upon derivation of the Michaelis-Menten term $Sn/(\beta + n)$, we observe that $\beta$ represents the ratio between the rate at which the receptors become empty and the rate at which the receptors become occupied. Hence a reasonable estimate is $\beta = 1$.

(b) **Diffusion coefficient** $d$. Sherratt & Murray [32] used estimated diffusion coefficients in their model of epidermal wound healing. For the diffusion of the cells they gave values of $3 \times 10^{-9}$ cm$^2$ s$^{-1}$, $3.5 \times 10^{-10}$ cm$^2$ s$^{-1}$, and $6.9 \times 10^{-11}$ cm$^2$ s$^{-1}$, and for the diffusion of the chemical in question they had $3.1 \times 10^{-7}$ cm$^2$ s$^{-1}$ and $5.9 \times 10^{-6}$ cm$^2$ s$^{-1}$. This gives a range for $d$ of $9.6 \times 10^{-1}$ to $1.2 \times 10^{-5}$. In their study of individual ECs, Stokes et al. [35] calculated a random motility coefficient of $(7.1 \pm 2.7) \times 10^{-9}$ cm$^2$ s$^{-1}$ for ECs migrating in a culture containing an angiogenic factor αFGF [13], heparin, and fetal calf serum. Taking $d_e$ as before, we obtain the range $1.4 \times 10^{-2}$ to $7.5 \times 10^{-4}$ for $d$. Therefore, in the numerical simulations, we chose a value for $d$ of $10^{-3}$, which lies in the middle of the range.

(c) **Rate of mitosis** $r$. For the rate of mitosis we took the range $0.04$ h$^{-1}$ (estimated by Sherratt & Murray [32]) to $0.956$ h$^{-1}$ (estimated by Stokes & Lauffenburger [34]). However, according to Bowersox & Sorgente [3], fibronectin inhibits cell
proliferation by 23%. Hence we have a range of 0.0308–0.043 h\(^{-1}\) for cell mitosis. We chose a value of 0.034, again in the middle of the range. Yamada & Olden [39] reported on the turnover rate of cell-surface fibronectin. Fibronectin has a generation time of 18 h with half remaining after 36 h. This gives a half life of 18 h and so the rate of decay is given by \(\gamma = \frac{1}{18} \ln 2 \cong 0.0385 \text{ h}^{-1}\). Hence we took mitotic parameter \(r = 0.034/0.0385 \cong 0.88\).

(d) **Haptotaxis coefficient a.** A Boyden chamber can be used to evaluate cell migration. Using this method, Ungari et al. [38] found that fibronectin mobilized ECs at a dose between 5 \(\mu\)g ml\(^{-1}\) to 20 \(\mu\)g ml\(^{-1}\). Bowersox & Sorgente [3] found that the maximum response by ECs was at 100 \(\mu\)g ml\(^{-1}\) and Terranova et al. [37] found that doses of fibronectin between \(10^{-8}\) M and \(10^{-10}\) M stimulated cell migration. However, this method fails to give information about the haptotaxis coefficient. Therefore we ran several numerical simulations and found that the best results were obtained with a value of \(a = 3.8\). Hence, using the values of \(\gamma\) and \(d_c\) which we found previously, this leads to a dimensional value of \(1.46 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1} \text{ M}^{-1}\) for the haptotactic coefficient.

(e) **Size of domain s.** The parameter value \(s\) represents the finite region of the capillary vessel that is affected by the TAF, so \(s\) will depend on the distance between the tumour and the capillary. As a scale reference, we will take \(s = 1\) when the tumour is at the maximum distance from the capillary but is still close enough for angiogenesis to take place [14]. From the summary of corneal implants reported by Balding & McElwain [2], the distance between the tumour and the host vessel should be in the range 0.8–2.5 mm in order to achieve neovascularization. Gimbrone et al. [14] observed that capillary sprouts did not grow until the tumour implant was placed within 2.5 ± 0.5 mm from the limbal vessels. Therefore we estimate our domain-size parameter \(s\) to be in the range 1–3. By increasing the size of the domain we can obtain a larger variety of pattern.

1.4 **Numerical simulations**

The nondimensionalized system of equations was solved using a subroutine available from the National Algorithms Group (NAG) library which integrates parabolic partial differential equations via the method of lines and Gear’s method. The parameter values used were as specified in the previous section and we had zero-flux boundary conditions. Initial conditions were taken to be small perturbations about the steady state (1, 0.5). As predicted by the linear stability analysis, the system evolved into a spatially inhomogeneous solution (see Figs. 3 and 4). The peaks in the cell density show that the cells have moved from their evenly distributed positions and have clustered together to form buds. These buds are assumed to sprout towards the tumour and become the primary capillary vessels in the vasculature of the tumour. Our simulation shows that two clusters form initially, which is consistent with observations made by Muthukkaruppan et al. [25] in their experiments with mouse cornea. The distribution of the chemical fibronectin and the distribution of the cells are similar. Thus the cells have moved towards areas of high chemical concentration.
Fig. 3. The density pattern of the ECs after being perturbed from an initially homogeneous steady state. Two peaks appear, indicating that two buds have formed. The parameter values are $d = 10^{-3}$, $a = 3.8$, $s = 3$, $r = 0.88$, and $N = 1$.

Fig. 4. The distribution of fibronectin after being perturbed about an initially homogeneous steady state. The pattern matches that of the ECs, indicating that the cells have moved towards the areas of high fibronectin concentration. The parameter values are $d = 10^{-3}$, $a = 3.8$, $s = 3$, $r = 0.88$, and $N = 1$. 

A TUMOUR-RELATED-ANGIOGENESIS MODEL
Since fibronectin is closely associated with cell adhesion, we assume that the main mechanism by which the cells move is via haptotaxis. The maxima in the chemical concentration coincide with the intensity of the TAF diffusing from the tumour; that is, the point of initial contact between the parent vessel and the TAF is the area where the first sprouts will be observed.

This model illustrates the principle of local activation and lateral inhibition [27]. Once a peak in fibronectin concentration has been established, ECs will actively move up the adhesive gradient, and hence the concentration of chemical in that area will increase. Even more cells will move into that particular area, and so on. Zones of inhibition will be created as cells move away from areas of low chemical concentration. These are indicated by the minima in Figs. 3 and 4. This highlights the importance of domain size to pattern formation. If the domain was too small, the cells would become saturated with fibronectin and they would not be able to detect gradients in the chemical/adhesive sites. Hence the zones of inhibition would disappear, and so would the pattern.

In Section 2 we focus upon ECs within the capillary sprout in order to investigate the branching process and the involvement of haptotaxis.

2. The branching of capillary sprouts

We now consider events concerned with the ECs after the formation of capillary buds. Having broken through the basement membrane, the buds elongate and form sprouts by recruiting ECs from the parent vessel. As the sprouts grow towards the tumour, secondary side branching occurs, whereby the sprouts themselves bud in a similar manner to the sprouts which developed from the parent vessel.

Two separate processes are involved in the creation of branching structures, such as capillary networks:

(i) the growth of branches; and
(ii) the splitting of one branch into two.

Since the resultant patterns depend upon the timing and the location of a splitting branch, there are often significant differences between branching structures, so that each one is distinct. Balding & McElwain [2] observed several similarities between fungal growth and neovascularization, and they subsequently based their model for capillary growth on a model of fungal colonies by Edelstein [8].

Similar structures are known to arise during the growth and development of bryozoans and fungi. Growth in bryozoans occurs at the tips of the branches, and the cells lying inside a growing tip secrete cuticle as the branch grows. The new cuticle is inserted directly into the existing cuticle, which spreads out, carrying cells and adhesive sites with it. The older cuticle is pushed outwards and calcifies, forming the outer layer of the branch. The direction of branch growth depends upon the location of the cells within the tip, and the splitting of a cell cluster into two coincides with the splitting of one branch into two. Hence the splitting of a cell cluster is the initial event which determines branching.

The model of bryozoan growth proposed by Goldwasser et al. [15] may be adapted
to describe other branching structures; in particular, it may be used as the basis for a model mechanism describing the branching of capillary sprouts during angiogenesis. The growing capillary-sprout tip is analogous to the growing tip of a bryozoan in that branching occurs when a cell cluster splits into two [21]. From experimental observations we can assume that during angiogenesis the ECs secrete a matrix consisting of fibronectin, laminin, and collagen IV [28], and the movement of the ECs is determined, at least in part, by the distribution of adhesive sites on this matrix. We can attribute the loss of matrix to the reformation of a basement membrane during the maturation of the newly formed capillary sprouts. It is known that ECs move up and down the new capillary sprout [28] and this motion cannot be explained either by directed cell motion alone, such as chemotaxis, or by random motility. However, it is known that convection plays a major role in the transport of tissue [24], and we assume that, as the matrix spreads out, the ECs are to some extent passively carried with it. Since proliferation occurs near the tip of the capillary sprout [1, 28], we do not need to include mitosis in the model.

2.1 Mathematical model

The model is constructed in a one-dimensional domain in order to focus attention on the EC clusters, rather than the emerging shape of the capillary-sprout tip. Let \( r(x, t) \) be the density of the matrix, \( a(x, t) \) be the density of the adhesive sites, and \( n(x, t) \) be the density of the ECs.

Mathematical models of tumour angiogenesis certainly rely on chemotaxis as the principal mechanism for cell motion [5, 34]. However, chemotaxis alone is insufficient to account for all the events associated with angiogenesis, especially those such as secondary side branching and anastomosis. Chemotaxis is implicit in this model, in that all the events of angiogenesis occur after the release of TAF by the tumour, and the cells at the tips of the sprouts are assumed to react to the chemotactic stimulus through migration and proliferation. It is these cells at the tips which primarily orientate the direction of the sprouts. In this section we focus attention on ECs behind the proliferating cells which are located near the sprout tip and as far back as the parent vessel (e.g. limbus), and hence it can be assumed, based on the experimental evidence cited previously, that the movement of the ECs (within the region of the sprout under consideration) is, in this case, governed by a combination of diffusion, haptotaxis, and convection.

The model is based on two processes [28]:

(i) The spreading of the matrix with the convection of ECs and adhesive sites with it.
(ii) The secretion of matrix and adhesive sites by the ECs.

The convection of cells and adhesive sites will be at the same rate as the spreading of the matrix. Assuming that the matrix spreads out at a constant rate, \( c \) say, and that the velocity is \( -r_x \), then the convective flux is proportional to \( -cr_x \); that is, the flux of the adhesive sites is \( J_a = -car_x \). For the source and sink terms, we assume linear production and decay of the matrix and adhesive sites, but, since proliferation of ECs occurs near the tip, we omit a cell-production term for the reasons cited
above. Hence the model equations are
\[ r_i = d_1 r_{xx} + \zeta n - \lambda r, \tag{12} \]
\[ a_i = d_2 a_{xx} + c(a r_x)_x + b n - \mu a, \tag{13} \]
\[ n_i = d_3 n_{xx} - h(na)_x + c(n r)_x, \tag{14} \]
where \( c \) is the rate at which the matrix spreads; \( \zeta \) is the rate of secretion of matrix per cell; \( \lambda \) is the loss of matrix per unit matrix; \( b \) is the rate of secretion of adhesive sites per cell; \( \mu \) is the decay of adhesive sites; \( d_1, d_2, d_3 \) are the diffusion constants; and \( h \) is the rate at which cells move up an adhesive gradient.

2.2 Nondimensionalization

Let \( N \) be the mean EC density throughout the capillary sprout; \( L_0 \) be the original length of the domain, that is, the length of the initial sprout bud; and \( L \) be the current length. The above system of equations can be nondimensionalized by making the following substitutions:

\[ n^* = \frac{n}{N}, \quad x^* = \frac{x}{L}, \quad t^* = \frac{\mu L^2_0 t}{L^2}, \quad a^* = \frac{ah}{\mu L^2_0}, \quad r^* = \frac{rc}{\mu L^2_0}, \]
\[ \lambda^* = \frac{\lambda}{\mu}, \quad \gamma^* = \frac{L^2}{L^2_0}, \quad \zeta^* = \frac{cN \zeta}{\mu^2 L^2_0}, \quad b^* = \frac{bhN}{\mu^2 L^2_0}, \quad d^* = \frac{d_i}{\mu L^2_0} \quad (i = 1, 2, 3). \]

Dropping the asterisks for notational convenience, the system of equations becomes
\[ r_i = d_1 r_{xx} + \gamma(\zeta n - \lambda r), \tag{15} \]
\[ a_i = d_2 a_{xx} + (ar)_x + \gamma(b n - a), \tag{16} \]
\[ n_i = d_3 n_{xx} - (n a)_x + (n r)_x. \tag{17} \]

To close the system we impose nonzero-flux boundary conditions for the matrix and the adhesive sites, but zero-flux boundary conditions for the cells. The reasoning behind these conditions is that the ECs stay entirely within the domain of the capillary sprout [28], whereas the matrix and the adhesive sites spread out beyond this domain so there is a certain amount of leakage at the boundaries. We therefore choose the boundary conditions
\[
\begin{align*}
d_1 r_x &= \mp k & \text{at } x = 0 \text{ and } x = 1, \text{ respectively,} \\
d_2 a_x + ar_x &= \mp l & \text{at } x = 0 \text{ and } x = 1, \text{ respectively,} \\\nd n_x - na_x + nr_x &= 0 & \text{at } x = 0 \text{ and } x = 1, \text{ respectively,}
\end{align*}
\tag{18}
\]
where \( k \) and \( l \) are positive constants representing the rate of loss of matrix and adhesive sites, respectively. We discuss the significance of the parameters \( k \) and \( l \) in Section 2.5.
2.3 Linear stability analysis

Assuming that the number of cells in the domain remains constant, that is, there is no proliferation in the region that we are considering, then we choose $n = 1$. The nontrivial spatially homogeneous steady state is $(\zeta/\lambda, b, 1)$. After linearizing about the steady state, we look for solutions of the form

$$ r = \begin{bmatrix} r - \frac{\zeta}{\lambda} \\ a - b \\ n - 1 \end{bmatrix}, \quad r_j \propto \exp(\sigma t + ikx) \quad (j = 1, 2, 3), $$

where $k$ is the wavenumber of the perturbation and $\sigma$ is the rate of growth of the perturbation with wavenumber $k$, as in Section 1.2.

The linearized system of equations takes the form $\sigma r = Ar$ where $A$ is a $3 \times 3$ matrix. The dispersion relation between $\sigma$ and $k$ is given by $\det[\sigma I - A] = 0$ (where $I$ is the identity matrix), which is

$$ \sigma^3 + \sigma^2[k^2(d_1 + d_2 + d_3) + \gamma(\lambda + 1)] 
+ \sigma[k^4(d_1 d_2 + d_2 d_3) + \gamma k^2(d_1 + \lambda d_2 + (1 + \lambda)d_3 - b + \zeta) + \gamma^2 \lambda] 
+ k^2[k^4d_1d_2d_3 + \gamma k^2[\zeta(b + d_2) + \lambda d_2d_3 + (d_3 - b)d_1] + \gamma^2[\zeta + \lambda d_3 - b]] = 0. \quad (19) $$

If $\text{Re}(\sigma) < 0$ then $\exp(\sigma t + ikx) \to 0$ as $t \to \infty$, so the system will be stable. If $\text{Re}(\sigma) > 0$ the perturbations will grow. The imaginary part of $\sigma$ gives rise to periodic solutions. When $k = 0$ the system is spatially homogeneous, and the dispersion relation becomes

$$ \sigma^3 + \gamma(\lambda + 1)\sigma^2 + \gamma^2 \lambda \sigma = 0, \quad (20) $$

which has a solution of $\sigma = 0$ and two negative roots. Hence the system is stable to spatially homogeneous solutions. When $k \neq 0$ the dispersion relation will either have three real roots or one real root and two complex conjugate roots. The root with the largest real part will determine the pattern which is formed. If this root is real and positive for a range of $k$, the initially uniform cell distribution will evolve into a spatially heterogeneous distribution. If this root is complex and its real part is positive then the perturbation will be oscillatory in nature.

In the following analysis, the theory used is standard for coefficients of polynomials such as Descartes rule of signs (see [24]). We will have a change in stability when $\sigma = 0$, that is, when $p(k^2) = 0$, where

$$ p(k^2) = a_1 k^4 + a_2 k^2 + a_3 $$

and

$$ a_1 = d_1 d_2 d_3, $$

$$ a_2 = \gamma [(d_3 - b)d_1 + \zeta(b + d_2) + d_2 d_3 \lambda], $$

$$ a_3 = \gamma^2 [\zeta + \lambda(d_3 - b)]. $$
If \( a_2 > 0 \) and \( a_3 > 0 \), then \( p(k^2) \) has no positive solutions for \( k^2 \). Consider \( a_3 < 0 \), that is, \( b > d_3 + \zeta/\dot{\lambda} \), then \( p(k^2) \) has one positive root. If \( a_3 > 0 \) but \( a_2 < 0 \), then \( p(k^2) \) has two positive roots, that is, when \( b < d_3 + \zeta/\dot{\lambda} \)
\[
 b(\zeta/\dot{\lambda} + d_1 - \zeta) > d_3(\zeta/\dot{\lambda} + d_1) + d_2\zeta + d_2d_3\dot{\lambda}.
\]

We will have exponential growth provided \( p(k^2) < 0 \). So if \( b > d_3 + \zeta/\dot{\lambda} \), we will have instability for \( k^2 \) in the range \( 0 < k^2 < k_0^2 \) and if
\[
 0 < b < d_3 + \zeta/\dot{\lambda}, \quad d_1 < \zeta,
\]
or if
\[
 \frac{d_1d_3 + d_2\zeta + d_2d_3\dot{\lambda}}{d_1 - \zeta} < b < d_3 + \frac{\zeta}{\dot{\lambda}}, \quad d_1 > \zeta,
\]
then we have instability for \( k_1^2 < k^2 < k_0^2 \) where \( k_{1,0}^2 \) are the roots of \( p(k^2) \).

Returning to the dispersion relation, we now consider the signs of the coefficients of the cubic equation, equation (19), that is, the signs of
\[
 A_1 = k^2(d_1 + d_2 + d_3) + \gamma(\dot{\lambda} + 1),
 A_2 = k^4[d_1(d_2 + d_3) + d_2d_3] + \gamma k^2[d_1 + \dot{\lambda}d_2 + (1 + \dot{\lambda})d_3 - b + \zeta] + \gamma^2\dot{\lambda},
 A_3 = k^2p(k^2).
\]

If \( A_3 < 0 \) then (19) has only one positive root, which will be real. If \( A_3 > 0 \) and \( A_2 > 0 \), the dispersion relation will have no positive roots. However, if \( A_3 > 0 \) and \( A_2 < 0 \) then the dispersion relation has two positive roots. So consider \( q(k^2) = 0 \) where \( q(k^2) = b_1k^4 + b^2k^2 + b_3 \) and
\[
 b_1 = d_1(d_2 + d_3) + d_2d_3,
 b_2 = \gamma[d_1 + \dot{\lambda}d_2 + (1 + \dot{\lambda})d_3 - b + \zeta],
 b_3 = \gamma^2\dot{\lambda}.
\]

If \( b_2 > 0 \) then \( q(k^2) \) has no positive roots. If \( b_2 < 0 \) then \( q(k^2) \) has two positive roots. So when
\[
 b > d_1 + \dot{\lambda}d_2 + (1 + \dot{\lambda})d_3 + \frac{\zeta}{\dot{\lambda}} + \zeta,
\]
\( q(k^2) < 0 \) for \( k_1^2 < k^2 < k_2^2 \), where \( k_{1,2}^2 \) are the roots of \( q(k^2) \).

There is another bifurcation point at which two of the real roots become complex. This point \( k_2^2 \) say, is given by solving
\[
 |\beta| = 2\alpha^{3/2},
\]
for \( k^2 \), where
\[
 \alpha = (\frac{1}{3}A_1)^2 - (\frac{1}{3}A_2), \quad \beta = 2(\frac{1}{3}A_1)^2 - (\frac{1}{3}A_1A_2) + A_3.
\]
The changes in the roots of the dispersion relation as $b$ increases can be summarized in the following way.

- For $0 < b < d_3 + \zeta / \lambda$ (provided $d_1 < \zeta$), the dispersion relation has only one positive root and hence we have instability for $k_A^2 < k^2 < k_B^2$.
- For $d_3 + \zeta / \lambda < b < d_1 + \lambda d_2 + (1 + \lambda) d_3 + \zeta / \lambda + \zeta$, the dispersion relation has only one positive root, and hence it is unstable for $0 < k^2 < k_B^2$.

For $b > d_1 + \lambda d_2 + (1 + \lambda) d_3 + \zeta / \lambda + \zeta$, the changes in the dispersion relation as $k^2$ increases are as follows.

- For $0 < k^2 < k_B^2$, the dispersion relation has one positive and two negative real roots.
- For $k_B^2 < k^2 < k_C^2$, all the roots are real and negative.
- For $k_C^2 < k^2 < k_1^2$, two roots are complex with negative real parts, and one root is real and negative.
- For $k_1^2 < k^2 < k_2^2$, the dispersion relation has one negative real root and two complex roots with positive real parts.
- For $k_2^2 < k^2$ the two complex roots have negative real parts.

For example, if we choose the parameters $d_1 = 1$, $d_2 = 1$, $d_3 = 10$, $\lambda = 20$, $\gamma = 15$, $\zeta = 10$, and let $10.5 < b < 241.5$, we would expect the system to evolve into a spatially heterogeneous state. We obtain the dispersion relation shown in Fig. 5 with $b = 155$. So for $0 < k^2 < 5.15$, we will have exponential growth.

![Fig. 5. A dispersion relation where $\sigma$ is real and positive for $0 < k^2 < 5.15$; hence we have instability for this range. The parameter values are $d_1 = 1$, $d_2 = 1$, $d_3 = 10$, $\lambda = 20$, $\gamma = 15$, $\zeta = 10$, $b = 155.$](image-url)
Another example is if we take \( b = 300 \). For this parameter set we obtain the dispersion relation shown in Fig. 6, where two of the roots of the dispersion relation have become complex with positive real part. In this case we would expect oscillating solutions, which correspond to ECs migrating up and down the sprout [28].

2.4 Estimation of parameters

Wherever possible, parameters were estimated from experimental papers. When this proved difficult, the parameters were chosen which yielded the best numerical results.

From [2] and [14], the tumour must be placed at a distance of 0.08–0.3 cm from the limbal vessel. Hence, we will take the initial length as \( L_0 = 0.001 \) cm and let \( L \) vary between 0.003 and 0.015 cm so that \( 9 < \gamma < 225 \).

Using the results of Yamada & Olden [39], we estimate the decay of the matrix to be \( 0.0385 \text{ h}^{-1} \). If we take \( \lambda = 20 \), then the decay of the adhesive sites \( \mu \) is \( 1.925 \times 10^{-3} \text{ h}^{-1} \). The choice of \( \lambda \) was arbitrary due to the lack of empirical data.

Yamada & Olden have reported [39] that fibronectin is relatively immobile with a diffusion coefficient less than \( 5 \times 10^{-12} \text{ cm}^2 \text{ s}^{-1} \). So taking the diffusion coefficient of the matrix and the diffusion coefficient of the adhesive sites to be \( 5.35 \times 10^{-13} \text{ cm}^2 \text{ s}^{-1} \), and taking the diffusion coefficient of the cells to be ten times higher, we have \( d_1 = d_2 = 1 \), and \( d_3 = 10 \). By making the diffusion of the cells lower than in the previous model, we are able to emphasize the other transport mechanisms which we believe to be more important.
Terranova et al. [37] found that doses of fibronectin between $10^{-8}$ and $-10^{-10}$ M stimulated cell migration, and Yamada & Olden [39] gave the generation time of fibronectin as 18 h. Assuming that $10^{-8}$ M of fibronectin is produced by $N$ cells in 18 h, where $N$ is the mean cell density, we estimate the rate of the secretion of the matrix to be $(10^{-8}/18)N^{-1} h^{-1}$ M. Again choosing $\zeta$ arbitrarily to be 10, we find that $c = 1.854738 \times 10^{-5} \text{cm}^2 \text{s}^{-1} \text{M}^{-1}$, which seems a reasonable estimate for the convection rate. Assuming that the secretion of the adhesive sites in ten times higher than that of the matrix and choosing $b = 155$ (example 1) and $b = 300$ (example 2), we obtain $h \approx 2.87 \times 10^{-5} \text{cm}^2 \text{s}^{-1} \text{M}^{-1}$ and $h \approx 5.56 \times 10^{-5} \text{cm}^2 \text{s}^{-1} \text{M}^{-1}$, respectively.

In theory the values for $k$ and $l$ could be determined experimentally. However, since there is no reliable empirical data, we will discuss the significance of these parameters in the next section.

2.5 Numerical simulations

We used the numerical scheme D03PGF available from the NAG library to solve the nondimensionalized system of equations. In principle, the model should display a variety of solutions, as predicted by the linear analysis. In practice, some of the solutions predicted by the linear stability analysis proved difficult to obtain.

The initial conditions used were the perturbations about the steady state

$$r = \frac{\zeta}{\lambda}, \quad a = b, \quad n = 1.$$  \hfill (21)

We experimented with small random perturbations of $O(0.01)$ as well as with a sine wave of small amplitude. This did not effect the resulting solution, but the numerical scheme ran best with random initial conditions. However, some of the solutions could only be obtained using initial conditions which were far away from the steady state. As stated earlier, we imposed nonzero-flux boundary conditions for the matrix and the adhesive sites, and zero-flux boundary conditions for the cells. This seems reasonable since we are only considering the splitting of an EC cluster rather than the actual formation and elongation of the branches. To model the formation of the branches themselves we would need to consider a moving domain.

The boundary conditions can be written as

$$d_1 r_x = \mp k,$$

$$d_1 d_2 a_x = \mp (d_1 l - ak),$$

$$d_1 d_2 d_3 n_x = \mp[d_1 l - (d_2 + a)k].$$  \hfill (22)

When written in this form, it is clear that our choice of $k$ and $l$ can effect the value of the solution for $n$ at each boundary. We assume that we must choose $k$ and $l$ such that

$$d_1 l - (d_2 + a)k > 0,$$  \hfill (23)

so that the sign of $n_x$ is consistent with the signs of $r_x$ and $a_x$ at each boundary.
Furthermore, the initial choice of \( a \) (i.e. our choice of the parameter \( b \)) can also effect the value of \( n_x \). In the examples that follow, we took \( d_1 = d_2 = 1 \). Hence our assumption is \( l > ak \) at \( x = 0, 1 \). In each of the numerical simulations that follow, we took \( k = 0.0001 \) and \( l = 1 \), since initially \( a = b \), where \( 155 \leq b \leq 300 \). We found that if we fixed \( l \) equal to 1 and varied \( k \) for \( k < 0.001 \) (i.e. (23) is satisfied), then the numerical solution for \( n \) did not change significantly. However, if we let \( k \) vary so that \( k > 0.001 \), then the numerical simulations broke down. Therefore, the solutions were sensitive to changes in the flux parameters, and so the boundary conditions are important in pattern formation.

Goldwasser et al. [15] found that one cell cluster forms when \( k_m = \pi \), and two cell clusters form when \( k_m = 2\pi \), \( k_m \) being the fastest growing wavenumber. By using the parameter values \( d_1 = 1, d_2 = 1, d_3 = 10, \lambda = 20, \gamma = 15, \zeta = 10, b = 155 \) in the NAG routine described above we obtained Fig. 7 where the density of the cells is greater at one end of the domain, indicating the formation of one EC cluster. We can increase the number of EC clusters formed by increasing our value of \( \gamma \), which is representative of the size of the domain. If we take \( \gamma = 60 \) we obtain two cell clusters, as in Fig. 8. Throughout our numerical investigation, solutions of this type were typical. It has been observed experimentally [12, 28] that the most common form in capillaries is Y- or T-shaped branching. According to the linear analysis, if we choose \( \gamma = 200 \), we should obtain three cell clusters. However, this solution was hard to capture and appeared to be transient. We obtained the solution in Fig. 9 using initial conditions.

![Fig. 7](image)

**Fig. 7.** An EC density profile obtained using parameter values \( d_1 = 1, d_2 = 1, d_3 = 10, \lambda = 20, \gamma = 15, \zeta = 10, b = 155 \). One EC cluster is formed towards \( x = 0 \). Note that \( x = 0 \) corresponds to a location close to a sprout tip but behind a proliferation compartment, and \( x = 1 \) corresponds to the location of the parent vessel (e.g. limbus).
for $a$ away from the steady state $a = b$; that is, initially we took $a = 200$. Hence, as
the domain increases (i.e. the capillary sprout grows), the more side branches there
are. Thus the number of side branches appearing depends on the size of the sprout.

By using the parameter values $d_1 = 1, d_2 = 1, d_3 = 10, \lambda = 20, \zeta = 10, b = 300$. We would expect oscillatory solutions. However, by using the initial
conditions of (21), we obtained a solution similar to that shown in Fig. 8. However, oscillating solutions could be obtained by using initial conditions away from the
steady state, that is, large perturbations. In the examples that follow we took initial
conditions for $a$ and $r$ to be $a = 200$ and $r = 0.5$. One oscillating solution, whereby
peaks of cells form from the initial conditions and momentarily merge before splitting
again, is illustrated in Fig. 10. This models the observation that the EC are continually
rearranging themselves, moving up and down the capillary sprout [28]. This also
reflects the transient behaviour of branching structures. Each time a cell cluster is
produced there is the possibility of a daughter branch forming, and hence no two
branching structures are exactly alike.

The linear stability analysis has shown that there is a critical value of the parameter
$b$ above which the solutions oscillate. Furthermore, by increasing the value of $\gamma$, we
increase the number of cell clusters formed. It is interesting to note that similar effects
can be obtained by varying the parameters $\zeta$ and $\lambda$. For example, an increase in $\zeta$
has the same effect on the dispersion relation as a simultaneous increase in $b$ and a decrease in $\gamma$. An increase in $\lambda$ has the exact opposite effect on the dispersion relation as an increase in $\zeta$. If we use the same parameters as in the last example but set $\zeta = 30$, then the dispersion relation is as shown in Fig. 11. From this we infer an increase in oscillatory behaviour. The numerical solution using these parameters (Fig. 12) has clusters of cells travelling to the right in a wave-like manner. Again, this demonstrates the persistent remodelling of the capillary network.

The difficulty in obtaining some of the predicted solutions in the numerical simulations indicates that the boundary conditions and initial conditions are very important in this model. Furthermore, the parameters must be chosen very carefully. Saunders & Ho [30] also found this to be true of a simple reaction–diffusion system modelling segmentation, and they concluded that this is a disadvantage of pre-pattern models in general, which do not have a mechanism for self-correction. A more robust model, such as a mechanochemical model where pattern formation occurs sequentially, would allow more freedom in the choice of parameters and would be less sensitive to the boundary and initial conditions. However, we would expect any model of branching to display transient behaviour in order to capture the uniqueness of every branching structure.
**FIG. 10.** A simulation showing cell clusters merging and then splitting again. The parameter values are $d_1 = 1$, $d_2 = 1$, $d_3 = 10$, $\lambda = 20$, $\zeta = 10$, $\gamma = 15$, $b = 300$. This models the observation of EC migration within the capillary sprout. Note that $x = 0$ corresponds to a location behind proliferating cells (near tip), and $x = 1$ corresponds to the parent vessel (e.g. limbus).

**FIG. 11.** A dispersion relation where the complex mode dominates. The parameter values are $d_1 = 1$, $d_2 = 1$, $d_3 = 10$, $\lambda = 20$, $\zeta = 30$, $\gamma = 15$, $b = 300$. 
3. Conclusions

The mathematical models for capillary-bud and capillary-side-branch formation in this paper have been developed using empirical data wherever possible, and the numerical solutions of the models are in good agreement with experimental observations of tumour angiogenesis.

It is our belief that all of the events associated with angiogenesis cannot be fully explained by chemotaxis alone. We have shown that haptotaxis is a potentially important mechanism in at least two of the stages of angiogenesis, and that it is worthy of further study. Schor & Schor [31] found that components of the ECM, such as fibronectin, increased the ability of ECs to react to angiogenic factors positively. Furthermore, they discovered that ECs did not react to an angiogenic stimulus when grown on denatured collagen or plastic materials. Haptotaxis has also been implicated in the formation of anastomoses [28]. Though this important event in angiogenesis is well documented, and it is known that capillary sprouts fuse together at their tips, the precise reason for this remains unexplained. Perhaps some mechanism which induces cell–cell adhesion may be involved. The role that haptotaxis may play could also suggest a potential anti-angiogenesis strategy by the development of drugs which reduce the property of ECs to adhere to the matrix.

Finally we note that the mathematical models that we have considered are minimal in that they include the most basic of equations concerning the chemical and cell distribution. We could elaborate the models further to include other factors such as the effect of traction forces on the matrix (see for example [23]). It may be possible
to gain some useful information on lumen formation and anastomosis by producing two-dimensional simulations of these models. We could also consider nonconstant diffusion/haptotactic coefficients. Nevertheless, these simple models have produced the desired results by encapsulating the formation of capillary buds and the side branching of capillary sprouts, and they have demonstrated the important potential role haptotaxis that may play in the angiogenic process.

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REFERENCES