



A Mathematical Analysis of a Model for Capillary Network Formation in the Absence of Endothelial Cell Proliferation

B. D. SLEEMAN

School of Mathematics

University of Leeds, Leeds LS2 9JT, U.K.

A. R. A. ANDERSON* AND M. A. J. CHAPLAIN

Department of Mathematics

University of Dundee, Dundee DD1 4HN, U.K.

(Received and accepted October 1998)

Communicated by W. Alt

Abstract—An analysis of a parabolic partial differential equation modelling capillary network formation is presented. The model includes terms representing cell random motility, chemotaxis, and haptotaxis due to the presence of chemical stimuli: tumour angiogenic factors and fibronectin. The analysis provides an underlying insight into mechanisms of cell migration which are crucial for tumour angiogenesis. Specific 1 and 2D examples are discussed in detail. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords—Angiogenesis, Endothelial cells, Chemotaxis, Haptotaxis, Sturm-Liouville problem.

1. INTRODUCTION

Angiogenesis (the formation of a capillary network of blood vessels from a pre-existing vasculature) is essential for the growth and development of solid tumours. Without such a network the tumour remains in a dormant avascular state. To facilitate angiogenesis the tumour secretes chemicals, collectively known as Tumour Angiogenic Factors (TAF) which induce neighbouring Endothelial Cells (EC) to degrade their basal lamina and to migrate into the Extracellular Matrix (ECM) towards the tumour. The ECM acts as a barrier to EC migration and has to be overcome in order for vascularization to be achieved. It is a fibrous material consisting of interstitial tissue, collagen fibre, fibronectin fibrils, fibronectin, and other components. Fibronectin enhances EC adhesion to the matrix and is also produced by the EC. Therefore, as well as moving in response to the chemical attractant TAF, EC migration is also regulated by the haptotactic effect of fibronectin (see [1] and the extensive references therein). Under these circumstances, the EC form capillary sprouts which continue to grow, branch, and form loops (anastomoses) through which blood eventually circulates. The branching increases dramatically as the tumour

*This work was supported by BBSRC Grant 94/MMI09008.

is approached. If EC mitosis is prevented the growth of capillaries is drastically reduced and results only in a restricted network being formed that never reaches the tumour [2]. Experimental results also show that disrupting fibronectin-specific receptors on EC can lead to failed vascularization [3].

In [4], Anderson and Chaplain discuss a possible mechanism to explain why capillary sprouts cease migrating towards the tumour in the absence of mitosis. In this paper, we provide a detailed analysis of the model providing a deeper understanding of the mechanisms involved.

2. THE MATHEMATICAL MODEL

In the absence of mitosis, the equation describing EC migration from [4] is,

$$\frac{\partial n}{\partial t} = D\Delta n - \operatorname{div}(\chi(c)n \operatorname{grad} c) - \rho_0 \operatorname{div}(n \operatorname{grad} f), \quad (1)$$

where $n(\underline{x}, t)$ is the EC density, D is the cell random motility coefficient, $\chi(c)$ is the chemotactic function (a measure of the sensitivity of EC receptors to TAF, c), f is the concentration of an adhesive chemical such as fibronectin, and ρ_0 is the (constant) haptotactic coefficient. Equation (1) is normally posed in a bounded domain Ω with no-flux boundary conditions on $\partial\Omega$, and is similar to the classic Keller-Segel-type model [5]. In [4], the analysis is restricted to one-space dimension in which $\Omega = [0, 1]$ and $c(x, t)$, $f(x, t)$ are assumed to be in quasi-steady state. In this case, (1) becomes

$$\frac{\partial n}{\partial t} = D \frac{\partial^2 n}{\partial x^2} - \frac{\partial}{\partial x} \left(\chi(c)n \frac{\partial c}{\partial x} \right) - \rho_0 \frac{\partial}{\partial x} \left(n \frac{\partial f}{\partial x} \right), \quad (2)$$

where c and f depend on x only. Furthermore, it is assumed in [4] that the chemotactic function $\chi(c)$ takes the form

$$\chi(c) = \frac{\chi_0}{1 + \alpha c}, \quad (3)$$

where χ_0 represents the maximum chemotactic response and α is a measure of the severity of desensitisation of EC receptors to TAF. A more detailed investigation of the problem in two-space dimensions with explicit time-dependent equations for the two chemical species (TAF, fibronectin) can be found in [6].

3. MODEL ANALYSIS AND SOLUTION

To begin with, we write (2) in the more general form

$$\frac{\partial n}{\partial t} = D \frac{\partial}{\partial x} \left[\frac{\partial n}{\partial x} - nH(x) \right], \quad (4)$$

where $H(x) = (\chi(c)/D) \frac{\partial c}{\partial x} + (\rho(f)/D) \frac{\partial f}{\partial x}$, (i.e., we make no particular assumptions regarding the chemotactic function $\chi(c)$ and the haptotactic function $\rho(f)$), subject to no-flux boundary conditions and initial conditions,

$$\frac{\partial n}{\partial x} - nH(x) = 0, \quad \text{at } x = 0, 1, \quad n(x, 0) = n_0(x) \geq 0. \quad (5)$$

We first of all remark that since $n_0(x)$ is nonnegative, it follows from the strong maximum principle [7], that $n(x, t)$ is nonnegative for all $t \geq 0$. Let us now solve (4) using separation of variables by setting $n(x, t) = T(t)N(x)$ to obtain

$$\frac{d}{dt}T + \lambda T = 0, \quad D \frac{d}{dx} \left[\frac{d}{dx}N - NH(x) \right] + \lambda N = 0. \quad (6)$$

From (6) it is clear that $T(t)$ is of the form $\exp(-\lambda t)$. If in (6) we make the substitution $N = \phi Y$, where $\phi = \exp(\int_0^x H(z) dz)$, then we arrive at the classical Sturm-Liouville problem for Y , namely

$$-D \frac{d}{dx} \left[\phi \frac{dY}{dx} \right] = \lambda \phi Y. \tag{7}$$

This problem has a complete orthonormal set of eigenfunctions $Y_i(x)$ in the weighted Hilbert space $L^2_\phi(0, 1)$ with eigenvalues λ_i ordered as, $0 = \lambda_0 < \lambda_1 \leq \lambda_2 \leq \dots$.

The eigenfunctions of (6) are then given by $N_i(x) = \phi(x)Y_i(x)$. Corresponding to $\lambda_0 = 0$ we have

$$N_0(x) = \exp \left[\int_0^x H(z) dz \right], \tag{8}$$

which is precisely the steady state solution given in [4] if we specialise the choice of $\chi(c)$ given by (3). That the λ_i ($i = 1, 2, \dots$) are positive follows in the standard way of multiplying (6) by N_i on $[0, 1]$ and using the boundary condition in (5). It is also easy to see that

$$\lambda_i \int_0^1 N_i dx = 0,$$

showing that the eigenfunctions $N_i(x)$ ($i = 1, 2, \dots$) have zero mean value. Again by standard expansion theory we know that the eigenfunctions form a complete orthonormal set in $L^2_{\phi^{-1}}(0, 1)$. Thus we can write

$$n(x, t) = A_0 N_0(x) + \sum_{i=1}^{\infty} A_i N_i(x) e^{-\lambda_i t}, \tag{9}$$

where the coefficients are determined by setting $t = 0$ and using the orthogonal properties of the eigenfunctions. Notice that as $t \rightarrow \infty$, $n(x, t) \rightarrow A_0 N_0(x)$ in (9), which is precisely the steady state discussed in [4].

4. EXAMPLES

As a first example, consider the case where $\chi(c) = \chi_0$, is a positive constant (i.e., $\alpha = 0$ in (3)) and the TAF concentration is $c(x) = m_1 x + 1 - m_1$, with $c(1) = 1$ at the tumour. $\rho(f) = \rho_0$ a positive constant, and the fibronectin concentration is $f(x) = 1 - m_2 x$, with $f(0) = 1$ at the blood vessel. This reflects the experimentally observed results of a decreasing fibronectin profile away from the parent vessel [8,9]. In this example,

$$H(x) = \frac{\chi_0}{D} m_1 - \frac{\rho_0}{D} m_2 \equiv \frac{\omega}{D} \tag{10}$$

and the associated eigenvalue problem becomes

$$D \frac{d}{dx} \left[\frac{d}{dx} N - N \frac{\omega}{D} \right] + \lambda N = 0,$$

that is

$$D \frac{d^2 N}{dx^2} - \omega \frac{dN}{dx} + \lambda N = 0, \quad \text{where } D \frac{dN}{dx} - N\omega = 0 \text{ at } x = 0, 1. \tag{11}$$

It is an easy matter to show that the eigenfunctions and eigenvalues for (11) are

$$N_0(x) = \exp \left(\frac{\omega}{D} x \right), \quad \lambda_0 = 0, \tag{12}$$

$$\begin{aligned} N_m(x) &= \exp \left(\frac{\omega}{2D} x \right) \left[\cos(m\pi x) + \frac{\omega}{2D\pi m} \sin(m\pi x) \right], & \lambda_m &= \frac{\omega^2}{4D} + m^2 \pi^2 D \\ &= \exp \left(\frac{\omega}{2D} x \right) \left(1 + \frac{\omega^2}{4m^2 \pi^2 D^2} \right) \cos(m\pi x + \beta_m), \end{aligned} \tag{13}$$

where $\tan \beta_m = -(\omega/2m\pi D)$ ($m = 1, 2, 3 \dots$). From the first two terms from (9) we may approximate $n(x, t)$ by,

$$n(x, t) \doteq A_0 \exp\left(\frac{\omega}{D}x\right) + A_1 \exp\left[\frac{\omega}{2D}x - \left(\frac{\omega^2}{4D} + \pi^2 D\right)t\right] \cos(\pi x + \beta_1). \tag{14}$$

From (14) we deduce the important fact that if $\omega = \chi_0 m_1 - \rho_0 m_2 < 0$, then the EC density $n(x, t)$ decays exponentially from the blood vessel ($x = 0$) to the tumour ($x = 1$). This is clearly achieved if $m_1/m_2 < \rho_0/\chi_0$. In the extreme case, this will happen if m_2 is large and/or when m_1 is small, implying a high concentration of fibronectin and/or a low concentration of TAF near $x = 0$ [8,9], i.e., EC migration will be restricted.

If $\alpha \neq 0$ then with the same choice of $c(x)$ and $f(x)$ problems (4),(5) can be solved in terms of confluent hypergeometric functions (see [10, p. 249]). However, due to the relative complexity of such functions, the expansion (9) is not particularly informative. Nevertheless, it is clear that desensitisation is related to lowering the chemotactic response and so the results above for $\alpha = 0$ are enhanced for $\alpha \neq 0$, as numerically demonstrated in [4].

Finally, we remark here that the second term in (14) shows that for $\omega < 0$, $n(x, t)$ has a wave-like component of the form

$$\exp\frac{\omega}{2D}\left[x - \left(\frac{\omega}{2} + 2\frac{\pi^2 D^2}{\omega}\right)t\right] \cos(m\pi x + \beta_m),$$

which represents a wave with decaying amplitude, moving back towards the blood vessel ($x = 0$) with speed $c = -(\omega/2 + 2(\pi^2 D^2/\omega))$.

To further illustrate the importance of haptotaxis in angiogenesis we consider a two-space dimensional example. Specifically, we consider (1) defined on the square region, $\Omega = \{x, y \in [0, 1] \times [0, 1]\}$, where the tumour occupies the region $x = 1, 0 \leq y \leq 1$, the parent blood vessel occupies the region $x = 0, 0 \leq y \leq 1$ and the regions $y = 0, y = 1, 0 \leq x \leq 1$ represent physical barriers. Thus along the boundary of the rectangular region we have the no-flux boundary conditions

$$\begin{aligned} D\frac{\partial n}{\partial x} - \chi(c)n\frac{\partial c}{\partial x} - \rho(f)n\frac{\partial f}{\partial x} &= 0, & \text{for } x = 0, 1 \text{ and } 0 < y < 1, \\ D\frac{\partial n}{\partial y} - \chi(c)n\frac{\partial c}{\partial y} - \rho(f)n\frac{\partial f}{\partial y} &= 0, & \text{for } 0 < x < 1 \text{ and } y = 0, 1. \end{aligned} \tag{15}$$

As above we take the quasi-steady state fibronectin concentration to have the form, $f(\underline{x}) = 1 - m_2 x$, $m_2 \leq 1$, and the TAF concentration to have the form, $c(\underline{x}) = 1 + m_1 x + m_3 y - (m_1 + m_3)$, $m_1 + m_3 \leq 1$, which might approximate the TAF gradient produced by a tumour centred on (1,1). Furthermore, we consider, as before, $\alpha = 0$ and $\rho(f) = \rho_0$. Note that the forms chosen for c and f are representative only and other choices can be made.

The model problem then becomes

$$\frac{\partial n}{\partial t} = D\Delta n - \omega\frac{\partial n}{\partial x} - \chi_0 m_3 \frac{\partial n}{\partial y}, \tag{16}$$

subject to (15) and $n(\underline{x}, 0) = n_0(\underline{x}) \geq 0$, where $\underline{x} = (x, y)$. By employing separation of variables $n(\underline{x}, t) = X(x)Y(y)T(t)$, we see that for some separation constants λ and μ ,

$$T(t) = \exp(-\lambda t), \tag{17}$$

$$D\frac{d^2 X}{dx^2} - \omega\frac{dX}{dx} + \mu X = 0 \tag{18}$$

and

$$D\frac{d^2 Y}{dy^2} - \chi_0 m_3 \frac{dY}{dy} + (\lambda - \mu)Y = 0. \tag{19}$$

Solving (18) subject to

$$D \frac{dX}{dx} - \omega X = 0, \quad x = 0, 1, \quad (20)$$

gives the same eigenvalue problem as the 1D case, resulting in the eigenfunctions and eigenvalues,

$$\begin{aligned} X_0(x) &= \exp\left(\frac{\omega}{D}x\right), & \mu_0 &= 0, \\ X_m(x) &= \exp\left(\frac{\omega}{2D}x\right) \left(1 + \frac{\omega^2}{4m^2\pi^2D^2}\right) \cos(m\pi + \beta_m), & \mu_m &= \frac{\omega^2}{4D} + m^2\pi^2D, \end{aligned} \quad (21)$$

where ω and β_m are as defined above. Setting $\Omega = \lambda - \mu$ and solving (19) subject to

$$D \frac{dY}{dy} - \chi_0 m_3 Y = 0, \quad y = 0, 1, \quad (22)$$

we obtain the following eigenfunctions and eigenvalues:

$$\begin{aligned} Y_0(y) &= \exp\left(\frac{\chi_0 m_3}{D}y\right), & \Omega_0 &= 0, \\ Y_l(y) &= \exp\left(\frac{\chi_0 m_3}{2D}y\right) \left(1 + \frac{\chi_0^2 m_3^2}{4l^2\pi^2D^2}\right) \cos(l\pi + \gamma_l), \end{aligned} \quad (23)$$

where $\Omega_l = (\chi_0^2 m_3^2 / 4D) + l^2 \pi^2 D$ and $\gamma_l = -(\chi_0 m_3 / 2l\pi D)$. Since $\lambda_{m,l} = \Omega_l + \mu_m$ we find

$$\begin{aligned} \lambda_{0,0} &= 0, & \lambda_{0,l} &= \frac{\chi_0^2 m_3^2}{4D} + l^2 \pi^2 D, & \lambda_{m,0} &= \frac{\omega^2}{4D} + m^2 \pi^2 D, \\ \lambda_{m,l} &= \frac{\omega^2 + \chi_0^2 m_3^2}{4D} + (l^2 + m^2) \pi^2 D, & & \text{with } l, m = 0, 1, 2, \dots, \end{aligned} \quad (24)$$

with the corresponding eigenfunctions

$$\begin{aligned} N_{0,0}(x, y) &= \exp\left(\frac{\omega}{D}x + \frac{\chi_0 m_3}{D}y\right), \\ N_{0,l}(x, y) &= \exp\left(\frac{\omega}{D}x + \frac{\chi_0 m_3}{D}y\right) \left(1 + \frac{\chi_0^2 m_3^2}{4l^2\pi^2D^2}\right) \cos(l\pi + \gamma_l), \\ N_{m,0}(x, y) &= \exp\left(\frac{\omega}{2D}x\right) \left(1 + \frac{\omega^2}{4m^2\pi^2D^2}\right) \cos(m\pi + \beta_m), \\ N_{m,l}(x, y) &= \exp\left(\frac{\omega x + \chi_0 m_3 y}{2D}\right) \left(1 + \frac{\omega^2}{4m^2\pi^2D^2}\right) \left(1 + \frac{\chi_0^2 m_3^2}{4l^2\pi^2D^2}\right) \cos(m\pi + \beta_m) \cos(l\pi + \gamma_l). \end{aligned} \quad (25)$$

These functions may now be used to write down the complete eigenfunction series representation for the EC density $n(x, y, t)$. As in the 1D example, the steady state solution is easily obtained from the solution to (25),(26) by setting $\lambda = 0$, since for large t the other terms decay to 0 (cf. (12),(24)). Therefore, as $t \rightarrow \infty$,

$$n(x, y) \rightarrow A_0 \exp\left(\frac{\omega x + \chi_0 m_3 y}{2D}\right). \quad (26)$$

Figure 1 shows a diagrammatic representation of the manner in which the sign of $\omega x + \chi_0 m_3 y / 2D$ affects the steady state solution (26). If, as in the 1D case, we have $\omega < 0$, then the resulting steady state decays exponentially from the blood vessel to the tumour. Provided $\omega x + \chi_0 m_3 y < 0$ (e.g., the solid line in Figure 1) no connection is made between the vasculature and the tumour, i.e., angiogenesis is not completed. However, if $\omega x + \chi_0 m_3 y \geq 0$ (e.g., the dashed line in Figure 1) then the steady state (26) predicts an exponential increase in EC density towards the tumour,

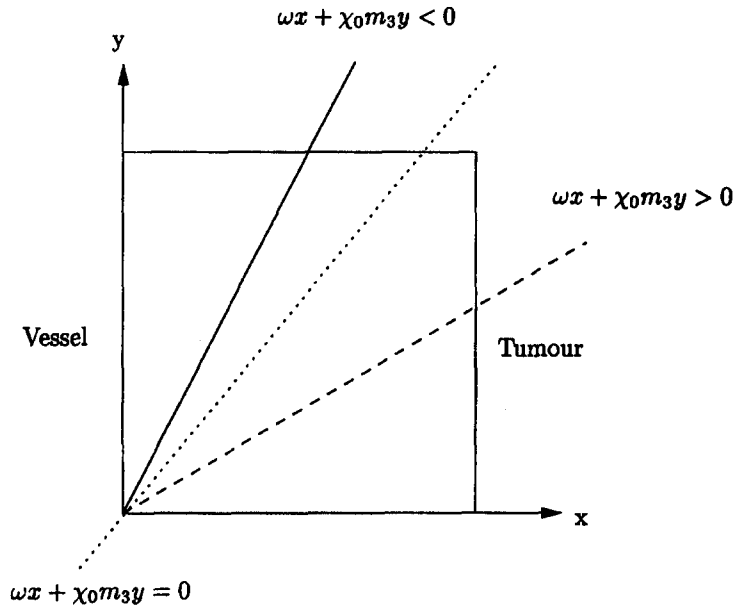


Figure 1. The effect of the sign of $\omega x + \chi_0 m_3 y$ on the steady state EC density. If $\omega x + \chi_0 m_3 y < 0$ then EC do not reach the tumour, however, if $\omega x + \chi_0 m_3 y > 0$ at least some EC will reach the tumour, and therefore, complete angiogenesis. Note, whether or not the line $\omega x + \chi_0 m_3 y = 0$ lies to the left or the right of line $y = x$ depends upon the magnitude of m_3 .

i.e., angiogenesis is completed. If $\omega > 0$ then there is always an exponential growth of EC towards the tumour, i.e., angiogenesis is always completed.

Of course, one should bear in mind that the analysis is based on particular choices of TAF and fibronectin concentration profiles. As in the 1D case, it is possible to write down a complete solution to the problem when $\alpha \neq 0$ in terms of confluent hypergeometric functions.

5. CONCLUSIONS AND DISCUSSION

We have presented an analysis of a mathematical model for capillary network formation in the absence of EC proliferation. As pointed out in [4] the model is experimentally verifiable and suggests an experiment whereby sources of TAF and fibronectin are placed at opposite ends of a square test-chamber. The initial distribution of EC is placed at the fibronectin end and the resulting spatio-temporal evolution of EC migration monitored.

Quite general forms of quasi-steady state TAF and fibronectin profiles can be used in this analysis. We have illustrated the effects in specific 1 and 2D examples, which emphasise the importance of the magnitude of the fibronectin and TAF concentration gradients in governing where and to what extent the capillary network fills the domain. Subsequently, the interplay between the chemotactic and haptotactic coefficients decides whether or not angiogenesis is completed.

REFERENCES

1. N. Paweletz and M. Knierim, Tumor-related angiogenesis, *Crit. Rev. Oncol. Hematol.* **9**, 197–242, (1989).
2. M.M. Sholley, G.P. Ferguson, H.R. Seibel, J.L. Montour and J.D. Wilson, Mechanisms of neovascularization. Vascular sprouting can occur without proliferation of endothelial cells, *Lab. Invest.* **51**, 624–634, (1984).
3. P.C. Brooks, A.M.P. Montgomery, M. Rosenfeld, R.A. Reisfled, T. Hu, G. Klier and D.A. Cheresh, Integrin $\alpha_v\beta_3$ antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels, *Cell* **79**, 1157–1164, (1994).
4. A.R.A. Anderson and M.A.J. Chaplain, A mathematical model for capillary network formation in the absence of endothelial cell proliferation, *Appl. Math. Lett.* **11** (3), 109–114, (1998).
5. E.F. Keller and L.A. Segel, Travelling bands of chemotactic bacteria: A theoretical analysis, *J. Theor. Biol.* **27**, 309–317, (1971).

6. A.R.A. Anderson and M.A.J. Chaplain, Continuous and discrete mathematical models of tumour—Induced angiogenesis, *Bull. Math. Biol.* **60**, 857–899, (1998).
7. M.H. Protter and H.F. Weinburger, *Maximum Principles in Differential Equations*, Springer-Verlag, (1984).
8. R.A.F. Clark, P. DellaPelle, E. Manseau, J.M. Lanigan, H.F. Dvorak and R.B. Colvin, Blood vessel fibronectin increases in conjunction with endothelial cell proliferation and capillary ingrowth during wound healing, *J. Invest. Dermatol.* **79**, 269–276, (1982).
9. S. Paku and N. Paweletz, First steps of tumor-related angiogenesis, *Lab. Invest.* **65**, 334–346, (1991).
10. A. Erdelyi, *Higher Transcendental Functions*, Volume 1, McGraw-Hill, (1953).