

Simulations of Endothelial Cells Clusters Migration in Angiogenesis

Tsung-Hsun Tsai*

*Associate Professor, Department of Mechanical Engineering, WuFeng University, TAIWAN. E-Mail: thtsai@wfu.edu.tw

Abstract—Tumor angiogenesis is the process that a capillary network is formed from a pre-existing vasculature in response to chemical stimuli (tumor angiogenic factors, TAF) secreted by a solid tumor. In order to supply a growing tissue such as a solid tumor with nutrients and oxygen, the formation of the capillary network (angiogenesis) are gradually growing towards the tumor by using the migration of endothelial cells driven by chemical stimuli gradient. In this study, a continuous and discrete mathematical model are considered to examine the migration of Endothelial Cells (ECs) and the formation of vessel capillary in response to chemical stimuli concentration gradient supplied by a solid tumor, respectively. Moreover, this study is also further to investigate the effects of a series of initial ECs clusters number releasing from parent vessel on ECs migration images, ECs density distribution and their patterns of capillary network when ECs migrate across the considered domain toward the tumor. In contrast to the present study, the effect of a series of initial ECs clusters number releasing from parent vessel on angiogenesis was not addressed in previous reports of others.

Keywords—Angiogenesis; Capillary Network; Chemical Stimuli Gradient; Endothelial Cells Clusters; Parent Vessel; Tumor Angiogenic Factors.

Abbreviations—Endothelial Cells (ECs); Extra Cellular Matrix (ECM); Tumor Angiogenic Factors (TAF); Vascular Endothelial Growth Factor (VEGF).

I. INTRODUCTION

ANGIOGENESIS is the formation of the capillary network from a pre-existing vasculature. It is an important biological process, not only under normally physiological conditions such as embryonic development and wound tissue healing, but also in a variety of diseases including solid tumor, diabetic retinopathy and rheumatoid arthritis [Folkman, 1995; Risau, 1997].

Tumor growth and metastasis are dependent on angiogenesis to supply the solid tumor with nutrients and oxygen. In order to supply a growing tissue such as a solid tumor with nutrients and oxygen, the capillary network (angiogenesis) are formed from a pre-existing vasculature in response to chemical stimuli that are secreted by a solid tumor. The secretions of chemical stimulus can be collectively called as Tumor Angiogenic Factors (TAF) [Folkman & Klagsbrun, 1987]. The concentration of TAF then can diffuse through the Extra Cellular Matrix (ECM) tissue creating the TAF gradient that attract Endothelial Cells (ECs) lining the parent vessel to thicken and produce a proteolytic enzyme (protease) which to degrade the parent vessel basement membranes and then penetrate the weakened basement membrane to form new capillary sprouts between the tumor and parent vessel. As the ECs continue to progress

toward the tumor through the ECM tissue, the ECs will produce fibronectin, a major component of ECM, to enhance cell adhesive capabilities to the matrix. It has been verified experimentally [Bowersox & Sorgente, 1982] that fibronectin stimulates directional migration of ECs by establishing an adhesive gradient.

In the past two decades, several mathematical models have been proposed to simulate some of the important features of angiogenesis. There are two principal classes of model used in this effort, namely continuous and discrete ones. In continuous model, details in the structure of capillary networks are neglected, wherein the macroscopic events such as evolution of EC density, migration and proliferation characteristics in response to chemical signals can be obtained [Anderson & Chaplain, 1998]. In discrete model, a probabilistic model based on stochastic differential equations in two space dimensions was proposed by Lauffenburger who described the motion of individual ECs [Stokes & Lauffenburger, 1991; Chaplain, 1996]. The random motility, chemotaxis (i.e., a response of the ECs to the gradients of TAF), haptotaxis (i.e., a complementary response to the gradients of fibronectin in the ECM, sprout branching and anastomosis were incorporated into discrete model; the microscopic world of capillary network that morphological similar to that observed in vivo can be captured. Sun et al.,

(2005) proposed a new formulation for modeling angiogenesis motivated by the results of Anderson et al., (1998). They define a capillary indicator function has binary values to describe the capillary network structure. Addison-Smith et al., (2008) developed a simple mathematical model of the siting of capillary sprouts on an existing blood vessel during the initiation of tumor-induced angiogenesis. They addressed the question of how unchecked sprouting of the chemical interaction between the proangiogenic and antiangiogenic factors. Billy et al., (2009) presented the multiscale model of tumor growth and angiogenesis to carry out a qualitative analysis of the effect of this treatment; they provided some indications about the best way to optimize a new anti-angiogenesis cancer treatment strategy. Cai et al., (2011) proposed a mathematical modeling system to investigate the dynamic process of tumor cell proliferation, death and tumor angiogenesis by fully coupling the vessel growth, tumor growth and blood perfusion. Tumor growth and angiogenesis are coupled by the chemical microenvironment and the cell-matrix interaction.

In this study, the hybrid models are adopted. We cite the work developed by Anderson and Chaplain [Anderson & Chaplain, 1998; Sleeman & Wallis, 2002]. The present study is the extension of the prior publication [Tsai, 2013]. Based on the need to further understand the details of microscopic features, the effects of a series of initial ECs clusters number releasing from parent vessel on ECs migration images, ECs density distribution and their patterns of capillary network are discussed. In contrast to the previous study, a single cell clusters across the domain toward a line source of tumor is added and variations of the profile of ECs density along the x -axis with a series of ECs clusters number moving across the domain is also considered.

II. CONTINUOUS MATHEMATICAL MODEL

2.1. Continuous Model

Figure 1 presents a schematic illustration of the tumor angiogenesis. As shown in figure 1, angiogenesis is initiated by the production of angiogenic factors from tumor cells, such as Vascular Endothelial Growth Factor (VEGF). Upon binding to its cognate receptors located on endothelial cells, such VEGF are sensed by ECs in preexisting parent vessels that subsequently produce proteases to dissolve the basement members, thereby allowing migration and proliferation of ECs into the direction of the stimulus. Degradation and invasion of extracellular matrix then follow. ECs assemble into a tubular structure. The process is completed by loop formation and vessel wall maturation [Michael et al., 2009; Pandya et al., 2006].

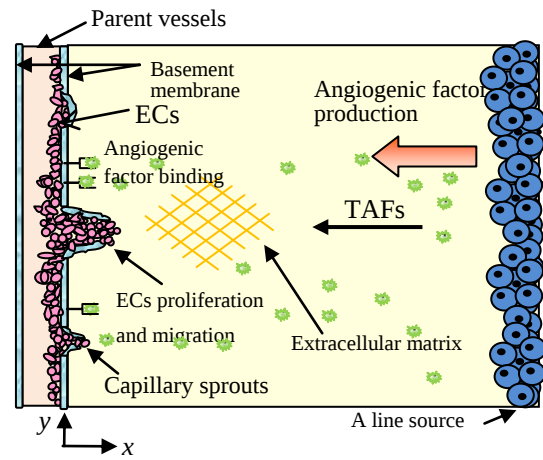


Figure 1: Schematic Illustration of the Tumor-Induced Angiogenesis

The continuous model process that is of interest to model is to examine the evolution of ECs density, migration and proliferation characteristics in response to chemical signals. As already described in figure 1, it is assumed that the motion of ECs is influenced by three factor, molecular diffusion (random motility), Chemotaxis in response to TAF gradients released by the tumor and haptotaxis in response to fibronectin gradients in the ECM. Let $n(x, y, t)$ denote the ECs density at spatial position x and y at time t , $f(x, y, t)$ denote the fibronectin concentration and $c(x, y, t)$ denote the TAF concentration. The migration of ECs from a parent vessel in response to chemical stimuli that are secreted by a solid tumor can be described using the following nondimensional equation initially proposed by Anderson and Chaplain (1998) and full details of the nondimensionalization can be found in Anderson and Chaplain (1998).

$$\frac{\partial n}{\partial t} = D \nabla^2 n - \nabla \cdot \left(\frac{X}{1 + \alpha c} n \nabla c \right) - \nabla \cdot (\rho n \nabla f) \quad (1)$$

$$\frac{\partial f}{\partial t} = \beta n - \gamma n f \quad (2)$$

$$\frac{\partial c}{\partial t} = -\eta n c \quad (3)$$

Where the coefficients D , X and ρ in Eq. (1) characterize the random, chemotactic and haptotactic cell migration, respectively (the chosen α parameter within the term of chemotactic in Eq. (1) is a positive coefficient). The two terms on the right-hand side of Eq. (2) describe the production and utilization of fibronectin species respectively as ECs migrate toward the tumor and it is noted that diffusion term does not appear in Eq. (2) due to fibronectin is bound to the ECM. The parameters of β and γ characterize the production rate and uptake rate by ECs cells as they migrate toward the tumor, respectively. The parameter of η in Eq. (3) is TAF consumption rate as ECs progress toward the tumor through the ECM. ∇ is the vector differential operator.

Equation (1) ~ (3) provides a general description of the migration of ECs interacted with TAF and fibronectin. To complete the model description, initial and boundary conditions need to be imposed upon the system.

2.2. Computational Geometry and Boundary Conditions

This system is considered to hold on a square spatial domain of side $L=1$ with the parent vessel located on the left boundary edge $x=0, y \in [0,1]$ and a line source of tumor located on the right boundary edge $x=1, y \in [0,1]$. First, it is assumed that the cells remain within the domain of tissue under consideration, therefore no-flux boundary conditions are assumed to hold on the boundary of the square domain as shown in figure 1.

$$\zeta \cdot (-D\nabla n + \frac{nX}{1+\alpha} \nabla c + \rho n \nabla f) = 0 \quad (4)$$

Where ζ is an appropriate outward unit normal vector.

The first event of tumor-induced angiogenesis is the secretion of TAF by the tumor cells. It is assumed that TAF profiles has been taken to be in pseudosteady state and has established an initial distribution of TAF in the considered domain. The initial condition for TAF concentration is specified by

$$c(x, y, 0) = \exp[-(1-x)^2 / \epsilon_1] \quad (5)$$

Where ϵ_1 is a positive constant. The choice of the form for TAF can be interpreted as a maximum concentration value of TAF at $x=1$ and then decreasing exponentially to minimum value at $x=0$.

Similarly, the initial condition for fibronectin concentration is choiced as follow:

$$f(x, y, 0) = k \exp(-x^2 / \epsilon_2) \quad (6)$$

Where $k < 1$ and ϵ_2 is a positive constant. The choice of the form for fibronectin can be interpreted as a minimum concentration value of fibronectin at $x=1$ and then increasing exponentially to maximum value at $x=0$.

After the TAF has reached the parent blood vessel at $x=0$, the ECs within the vessel form into a few cell clusters which eventually become sprouts. For the initial configuration of ECs along the boundary $x=0$ is specified by

$$n(x, y, 0) = 0.9 \exp[-(x-0)^2 / \epsilon_3 + (y-y_i)^2 / \epsilon_3] \quad (7)$$

Where ϵ_3 is a positive constant involved the size of ECs cluster at the initial position of parent vessel. y_i is the position of ECs clusters formed along the y -axis at $x=0$, where $i = 1, 2, 3, \dots, n$.

The simulation results for the variation of chemical stimuli concentration gradient of TAF and fibronectin during the migration of ECs toward the tumor can be achieved by using the system of equations (1)-(3) with boundary and initial conditions (4)-(7) if proper physiological parameters for each species are provided.

2.3. Parameter Values

To set up a realistic simulation, the physiological parameters are given as following. It is worthy mention that all the physiological parameter values used in the simulation are mostly obtained from Anderson and Chaplain (1998). Specifically, these are identical values as Anderson and Chaplain (1998) for the following constants

$$D = 0.00035, \alpha = 0.6, X = 0.38, \rho = 0.34, \beta = 0.05, \\ \gamma = 0.1, \eta = 0.1, \epsilon_1 = 0.45, \epsilon_2 = 0.45, \epsilon_3 = 0.002, \\ k = 0.75$$

The time parameter was normalized as $\tilde{t} = t/\tau$ with $\tau = L^2/D_c$, where $L = 2$ mm is the length of the domain and $D_c = 2.9 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ is taken as the diffusion coefficient for TAF. The estimates for L and D_c give the timescale $\tau = L^2/D_c$ as 1.5 days. Details of the parameter normalization are given in Anderson and Chaplain (1998).

III. SIMULATION RESULTS OF CONTINUOUS MODEL

The system of partial differential equation (1) and ordinary equations (2)-(3), subject to the boundary and initial conditions (4)-(7) can show the morphological patterns of ECs migration and proliferation in response to TAF and fibronectin. Owing to the model of these equations are very complex so that numerical simulations are required. Equations (1)-(7) are then coupled and solved using COMSOL Multiphysics 3.4 software in this study.

Migration and proliferation of CE is stimulated by TAF and fibronectin. The TAF and fibronectin sources are given by Eq. (5) and (6) respectively. The initial concentration profile of TAF and fibronectin in the ECM is plotted as shown in figure 2. It can be seen that a maximum concentration value of TAF is at $x=1$ and then decreasing exponentially to minimum value at $x=0$. In contrast to TAF, a minimum concentration value of fibronectin at $x=1$ and then increasing exponentially to maximum value at $x=0$. The TAF concentration profile approximates a gradient produced by a line of tumor.

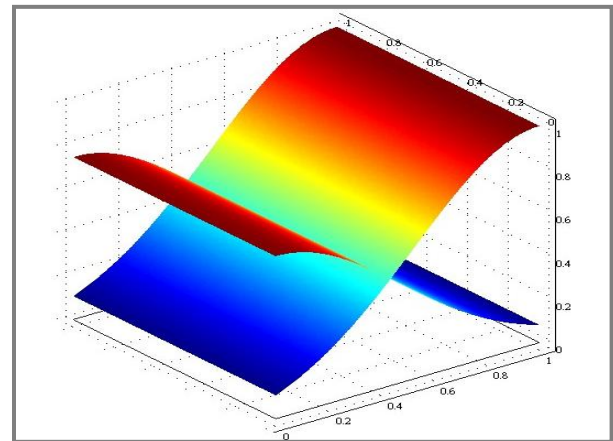


Figure 2: Initial Concentration Profile of TAF and Fibronectin

3.1. Single Cell Clusters Release from Parent Vessel

In order to examine the relative importance of chemotaxis and haptotaxis in details, migration of single ECs clusters across the domain toward a line source of tumor is first considered. It is assumed that the single cell clusters is formed on the parent vessel at the position of $y=0.5$.

The simulation results show that the initial configuration of the single ECs clusters at $t=0$ appearing as a small circular shape is shown in figure 3. As time proceeding, the ECs clusters migrate up the TAF gradient toward the tumor and tend to diffuse into the surrounding ECM. The clusters of ECs density distribution at different time step appear as a

crescent-like shape and continue to develop and progress. From the results of ECs density profile as shown in figure 3 reflect that of the migration of ECs interacted with TAF and fibronectin is capable of producing lateral movement of the cells.

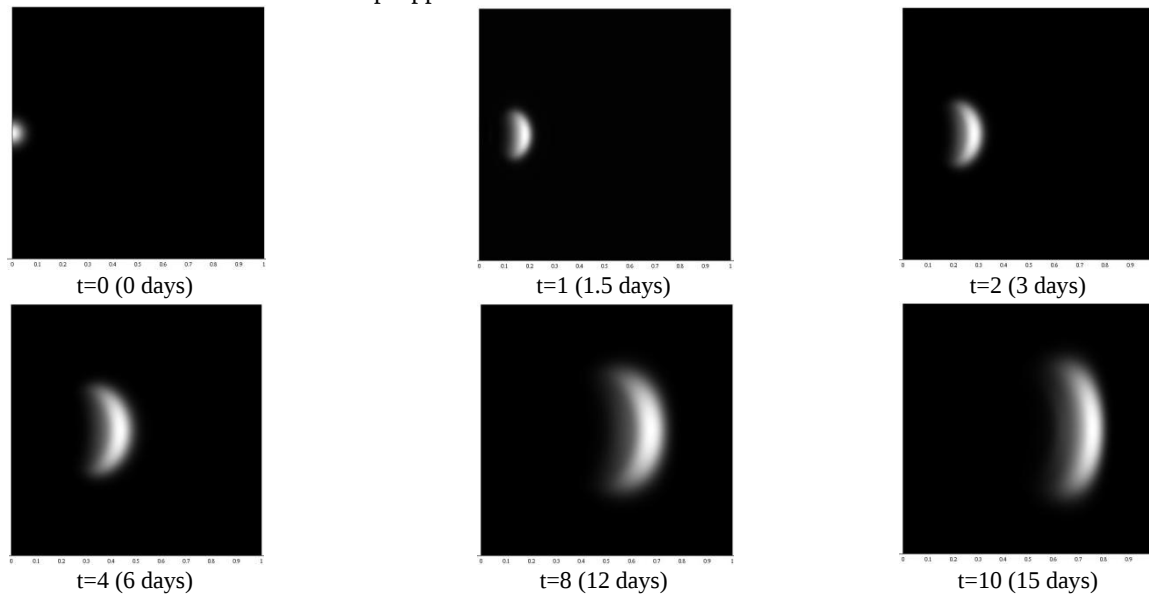


Figure 3: Single ECs Clusters are Formed on the Parent Vessel at the Position of $y=0.5$ and Migrate Toward the Tumor

Figure 4 show the variations of the profile of ECs density along the x -axis at the cross section of $y=0.5$. Profiles of ECs density are plotted at times $t=0,1,2,\dots,10$. It can be seen in figure 4 that a peak of ECs density moving across the domain towards the tumor, and there is a decline in ECs density at the cross section of $y=0.5$ with time. This is due to the fact that ECs diffuse into the considered domain as ECs migrate toward the tumor. This lateral motion of diffusion of ECs results in the decreasing of the peak value of the ECs density. It is noted that the qualitative form of the simulation are wave-like, therefore it can be estimated that the speed of the wave by examining the numerical solution. Hence, it is seen in figure 4 that the migration of ECs clusters velocity is becoming slowly advancing toward the tumor.

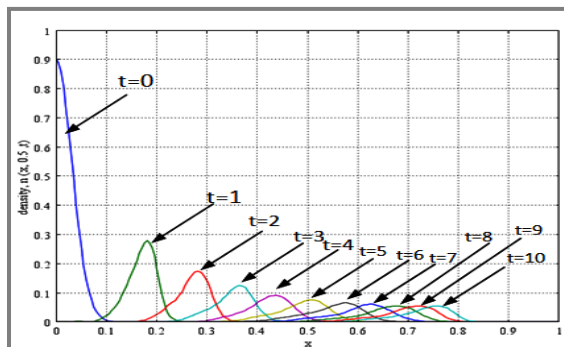


Figure 4: Variations of the profile of ECs density along the x -axis with regular time intervals at the cross section of $y=0.5$ as single ECs clusters moving across the domain towards the tumor

3.2. Three Cell Clusters Release from Parent Vessel

In the second set of simulations is three ECs clusters releasing from parent vessel (all parameters having the same values as used for figure 3). It is assumed that the three ECs clusters are formed on the parent vessel at the position of $y=0.2, 0.5$ and 0.8 as shown in figure 5. In the early stage of simulation, there are no change in the behavior and shape of the individual cell clusters (see figure 3, $t=0$ and $t=1$). As time proceeding, the ECs migrate up the TAF gradient toward the tumor and tend to diffuse into the surrounding ECM. By $t=4$ it can be seen that the three separate ECs clusters have joined to form a continuous band of ECs density. Subsequently at $t=8$, the three ECs clusters have overlapped. At the final time, $t=10$, the three ECs density distribution has formed into a band and very slowly migrates toward the tumor.

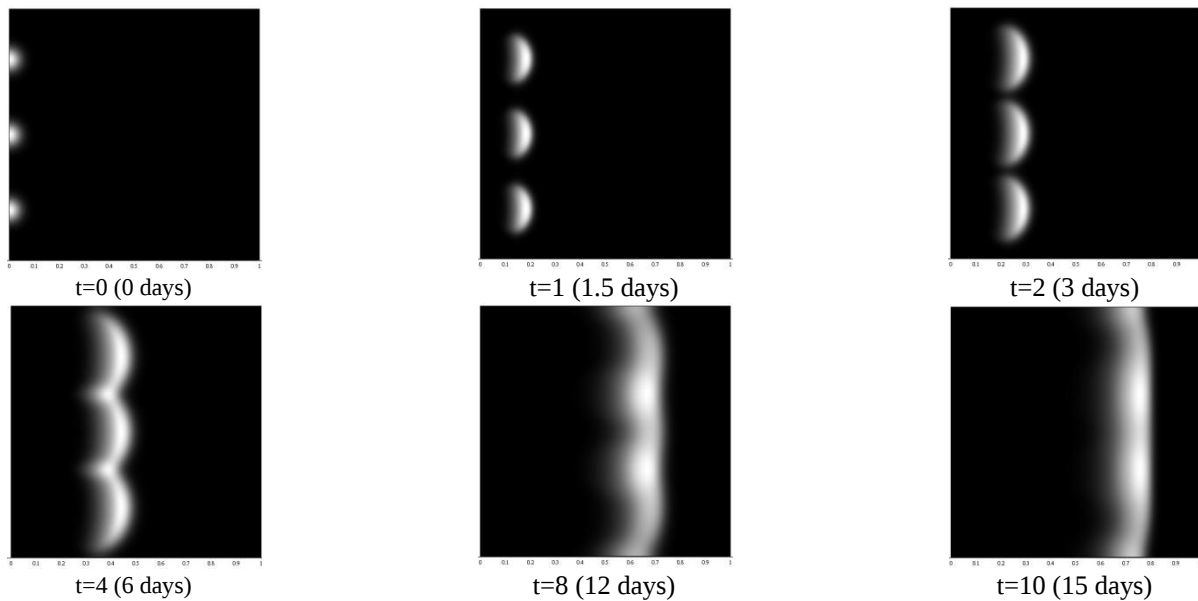


Figure 5: Three ECs clusters are formed on the parent vessel at the position of $y=0.2, 0.5$ and 0.8 and migrate towards the tumor

Figure 6 show the variations of the profile of ECs density along the x -axis at the cross section of $y=0.5$ as three cell clusters moving across the domain towards the tumor. It can be seen that the height of the peak of the ECs density decreases at $t<8$ and then a little increase at $t=9$ and $t=10$. This is because there are three ECs clusters within the domain resulting in the higher peak value of ECs density accumulated at the band. Except that, the profile of ECs density along the x -axis at the cross section of $y=0.5$ are not significantly different comparing with figure 4.

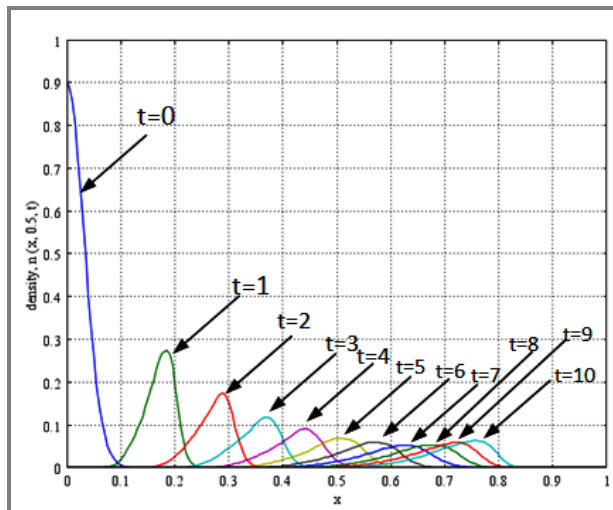


Figure 6: Variations of the profile of ECs density along the x -axis with regular time intervals at the cross section of $y=0.5$ as three ECs clusters moving across the domain towards the tumor

3.3. Five Cell Clusters Release from Parent Vessel

In order to further understand the patterns of ECs density distribution with more numbers of ECs clusters releasing from parent vessel, the final set of simulations is five ECs clusters releasing from parent vessel. It is assumed that the five ECs clusters are formed initially on the parent vessel at the position of $y=0.1, 0.3, 0.5, 0.7$ and 0.9 respectively. The initial configuration of the five ECs clusters is shown in figure 7 at $t=0$ (all parameters also having the same values as used for figure 3). As seen in figure 7, the ECs migrate up towards the tumor with chemotaxis and haptotaxis. It can be seen clearly that the five separate ECs clusters have joined to form a continuous band of ECs density at $t=1$ and have overlapped at $t=2$. Obviously, at $t=4$, the five ECs density distribution has become a band. At the final time $t=10$, the five ECs density distribution has diffused uniformly into the surrounding ECM and formed a band which is slowly advancing toward the tumor with the highest ECS density at the leading edge.

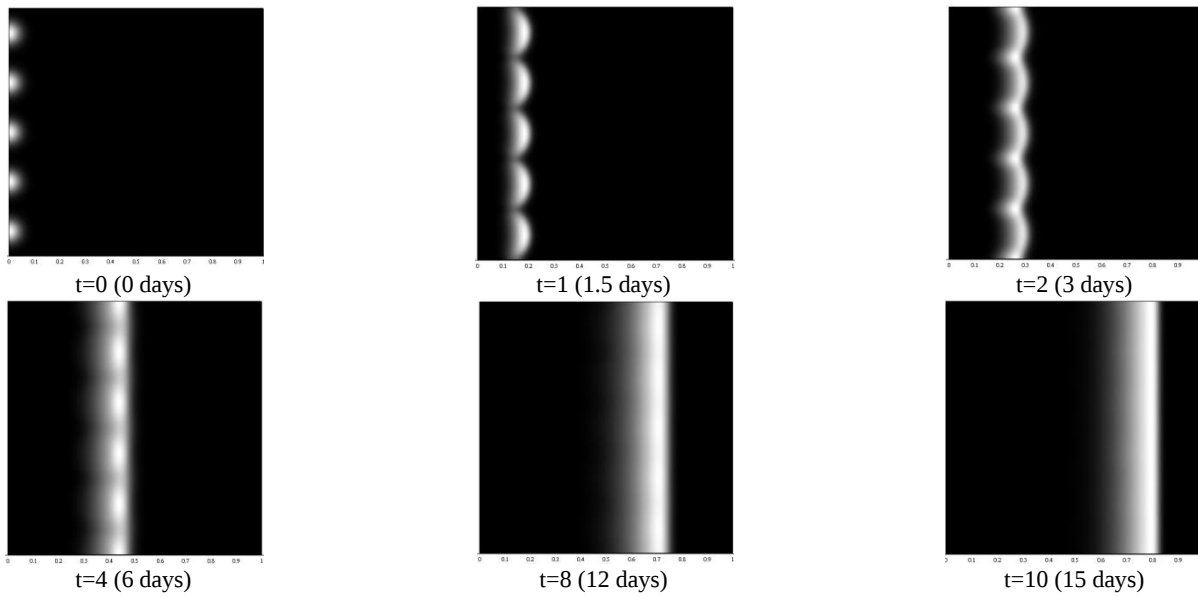


Figure 7: Five ECs clusters are formed on the parent vessel at the position of $y=0.1, 0.3, 0.5, 0.7$ and 0.9 and migrate towards the tumor

Comparing the single and three ECs clusters releasing from parent vessel case, the peak value of the ECs density along the x -axis at the cross section of $y=0.5$ in figure 8 is now more pronounced than those in figure 4 and figure 6. It is also noted that the qualitative form of the simulation is wave-like, therefore it can be estimated that the speed of the wave by examining the numerical solution. Hence, it is seen in figure 8 that the migration of ECs clusters velocity is faster advancing toward the tumor than the single and three cell clusters releasing from parent vessel.

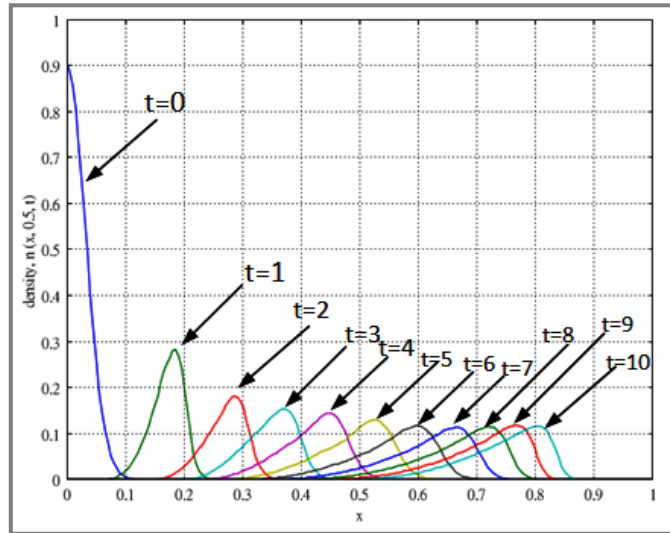


Figure 8: Variations of the profile of ECs density along the x -axis with regular time intervals at the cross section of $y=0.5$ as five ECs clusters moving across the domain towards the tumor

IV. DISCRETE MATHEMATICAL MODEL

4.1. Discrete Model

In order to capture the specific features of capillary network growth, such as branching and anastomosis (loop formation), it is needed to use the discretized form of the system of

partial differential equation (1) and ordinary equations (2), (3). The discrete model is solved using a standard finite difference scheme, and implemented on MATLAB. The discrete model allows us to track the motion of individual ECs located at the capillary sprout tips and the subsequent formation of capillaries. Therefore, the continuous system of PDE and ODE equations were discretised by using the standard Euler finite difference approximations to obtain the transition probabilities as below:

$$n_{i,j}^{q+1} = n_{i,j}^q \cdot P_0 + n_{i+1,j}^q \cdot P_1 + n_{i-1,j}^q \cdot P_2 + n_{i,j+1}^q \cdot P_3 + n_{i,j-1}^q \cdot P_4 \quad (8)$$

$$f_{i,j}^{q+1} = f_{i,j}^q \cdot (1 - k \cdot \gamma \cdot n_{i,j}^q) + k \cdot \beta \cdot n_{i,j}^q \quad (9)$$

$$c_{i,j}^{q+1} = c_{i,j}^q \cdot (1 - k \cdot \eta \cdot n_{i,j}^q) \quad (10)$$

Where i, j and q are positive parameters which specify the location on the grid and the time step, i.e., $x = ih, y = jh, t = qk$ and

$$P_0 = 1 - \frac{4kD}{h^2} + \frac{k}{4h^2} \cdot \left[\frac{X \cdot \alpha}{(1 + \alpha c_{i,j}^q)^2} \right] \cdot \left[(c_{i+1,j}^q - c_{i-1,j}^q)^2 + (c_{i,j+1}^q - c_{i,j-1}^q)^2 \right]$$

$$- \frac{k}{4h^2} \cdot \left[\frac{X}{1 + \alpha c_{i,j}^q} \right] \cdot \left[c_{i+1,j}^q + c_{i-1,j}^q - 4c_{i,j}^q + c_{i,j+1}^q + c_{i,j-1}^q \right]$$

$$- \frac{k\rho}{4h^2} \cdot \left[f_{i+1,j}^q + f_{i-1,j}^q - 4f_{i,j}^q + f_{i,j+1}^q + f_{i,j-1}^q \right]$$

$$P_1 = \frac{kD}{h^2} - \frac{k}{4h^2} \cdot \left(\frac{X}{1 + \alpha c_{i,j}^q} \right) \cdot (c_{i+1,j}^q - c_{i-1,j}^q) - \frac{k\rho}{4h^2} \cdot (f_{i+1,j}^q - f_{i-1,j}^q)$$

$$P_2 = \frac{kD}{h^2} + \frac{k}{4h^2} \cdot \left(\frac{X}{1 + \alpha c_{i,j}^q} \right) \cdot (c_{i+1,j}^q - c_{i-1,j}^q) + \frac{k\rho}{4h^2} \cdot (f_{i+1,j}^q - f_{i-1,j}^q)$$

$$P_3 = \frac{kD}{h^2} - \frac{k}{4h^2} \cdot \left(\frac{X}{1 + \alpha c_{i,j}^q} \right) \cdot (c_{i,j+1}^q - c_{i,j-1}^q) - \frac{k\rho}{4h^2} \cdot (f_{i,j+1}^q - f_{i,j-1}^q)$$

$$p_4 = \frac{kD}{h^2} + \frac{k}{4h^2} \cdot \left(\frac{X}{1 + \alpha c_{i,j}^q} \right) \cdot (c_{i,j+1}^q - c_{i,j-1}^q) + \frac{k\rho}{4h^2} \cdot (f_{i,j+1}^q - f_{i,j-1}^q)$$

The five coefficients P_0 to P_4 be used from Eq. (8) are to generate the motion of individual ECs. It can be seen from above expressions that these coefficients involve the effects of random, chemotactic and haptotactic movement and strongly depend upon the local chemical environment (fibronectin $f(x, y, t)$ and TAF $c(x, y, t)$). The migration of an individual ECs located at the tip of a sprout is determined by the set of coefficients P_0 to P_4 which are proportional to the probabilities of the ECs being stationary (P_0), or moving left (P_1), right (P_2), up (P_3), down (P_4). The transition probabilities are then used to define probability intervals as follow.

$$R_0 = 0 \sim p_0$$

$$R_1 = p_0 \sim (p_0 + p_1)$$

$$R_2 = (p_0 + p_1) \sim (p_0 + p_1 + p_2)$$

$$R_3 = (p_0 + p_1 + p_2) \sim (p_0 + p_1 + p_2 + p_3)$$

$$R_4 = (p_0 + p_1 + p_2 + p_3) \sim (p_0 + p_1 + p_2 + p_3 + p_4)$$

We then generate a random number between 0 and 1, and depending on the range in which the number falls, the ECs under consideration will tend to remain stationary (R_0), or move left (R_1), right (R_2), up (R_3), down (R_4). The larger a particular probability interval is, the greater the probability that such an interval will be selected.

4.2. Rules for Branching and Anastomosis

In order to implement capillary splitting, anastomosis, and proliferation, the processes of branching (formation of new sprouts from existing sprout tips) and anastomosis (formation of loops by fusion of two colliding capillary sprouts) are incorporated into the discretized form of the model.

The main features of the process of the movement of ECs in the formation of the capillary network are (this is similar to the work by Anderson and Chaplain (1998)).

- (1) For branching, the probabilities for an existing sprout branching increases with the local TAF concentration.
- (2) For anastomosis, if two sprouts collide as they grow, only one of them is allowed to keep growing (the choice of which is random),
- (3) If a sprout tip meets another sprout, they fuse to form a loop.

4.3. Simulation Results of Discrete Model

All the simulations of the discrete model are carried out on a 200×200 grid, which is a discretization of the unit square domain, $[0,1] \times [0,1]$, with a space step of $h=0.005$. The grid is partitioned in such a way that each cellular grid element corresponds in size with the actual biological ECs of interest (10–20 μm ; Anderson and Chaplain (1998)). A discrete form of the no-flux boundary condition (4) was imposed on the

square grid, restricting the ECs to within the considered domain. The initial conditions in all simulations are given by Eq. (5) and (6).

4.3.1. Single Cell Clusters Release from Parent Vessel

As with the continuous model simulation we will initially consider the discrete simulations of capillary network formation with single ECs clusters releasing from parent vessel as shown in figure 9. Clearly, the capillary network are migrating from the parent vessels ($x=0$) toward a line source of tumor. As the time increases, the sprouts begin to branch and spread into the domain. The dendritic structure of capillary network is sharply captured. As the sprouts progress and near the tumor, there is more amount of branching and the anastomosis also can be seen clearly in the simulation pattern of capillary network. Comparing this result with the continuous equivalent in figure 3, it can be seen that the sprout progression matches well with the movement of the high area of ECs density.

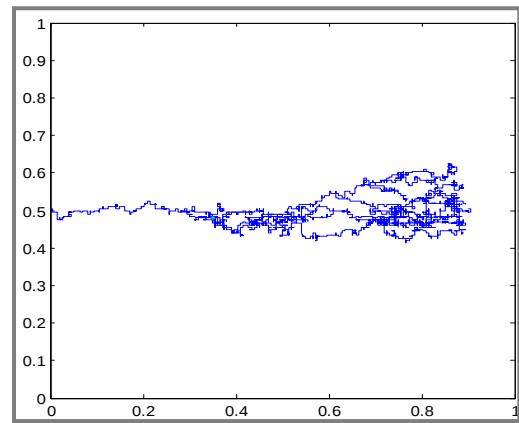


Figure 9: Capillary Network with Single ECs Clusters Release from Parent Vessel

4.3.2. Three Cell Clusters Release from Parent Vessel

The simulations of capillary network formation with three ECs clusters releasing from parent vessel is presented in figure 10. Clearly, the capillary network are migrating from the parent vessels ($x=0$) toward a line source of tumor. In the early stages, when the capillaries are still near to the parent vessel, there is little vessel branching. As the time further increases, there is widespread vessel branching and the formation of many closed loops, or anastomoses in response to the local TAF and fibronectin gradient. However, the pattern of capillary network developed due to the migration of each ECs clusters toward the tumor have significantly different structure. This due to the formation of capillary network is determined by the set of coefficients P_0 to P_4 which these coefficients involve the effects of random, chemotactic and haptotactic movement and strongly depend upon the local chemical environment (fibronectin and TAF concentrations).

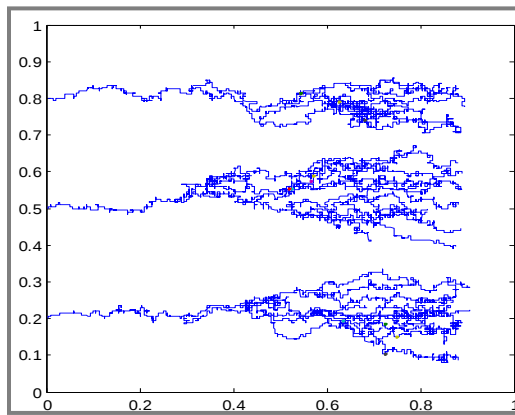
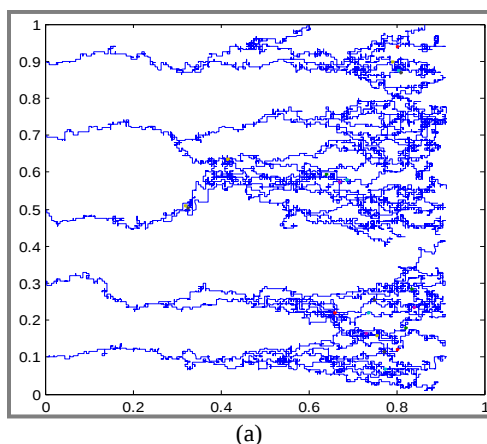


Figure 10: Capillary Network with Three ECs Clusters Release from Parent Vessel

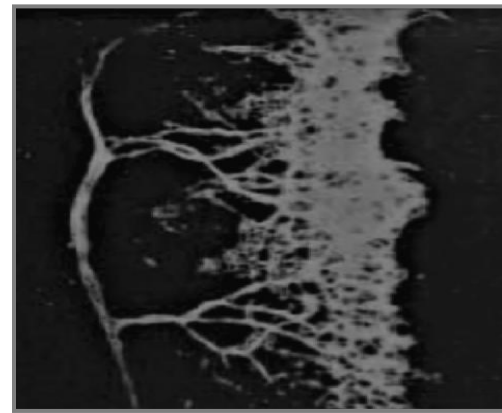
4.3.3. Five Cell Clusters Release from Parent Vessel

Figure 11 (a) shows the simulations of capillary network formation and vascular architecture with five ECs clusters releasing from parent vessel. It is seen in figure 11 (a) the stochastic nature of each of the five sprout trajectories as they progress towards the tumor and the migratory path taken by each vessel is essentially independent of its neighbors. As times further increases, some degree of sprout branching and local anastomosis has already taken place for all five sprouts. At position of $x=0.4$, vessels 3 and 4 have formed an anastomosis. Comparing this result with the continuous equivalent in figure 7, it can be seen that the sprout progression matches well with the movement of the high area of ECs density. It is seen in figure 11 (a) that local anastomosis increases considerably with increased sprout branching in the distal region of capillary network and the individual vascular trees rapidly connect with one another. It can also be observed that as the tumor is approached, the sprouts coalesce and form the ‘brush border’.

The results show qualitative resemblance to experimental vascular networks, such as this shown in figure 11(b) (though it should be noted that the geometry, initial conditions and chemotactic gradients in these experiments are not the same as in the simulations).



(a)



(b)

Figure 11: (a) Capillary network with five ECs clusters release from parent vessel. (b) Reproducing images from Asahara et al. (1998) show angiogenic response in a mouse cornea 6 days after implantation with a pellet containing VEGF. The image also displays the brush-border effect associated with repeated capillary branching

V. CONCLUSIONS

In this study, the continuous and discrete mathematical models are considered to examine the migration of endothelial cells (ECs) and the formation of vessel capillary in response to chemical stimuli concentration gradient supplied by a solid tumor.

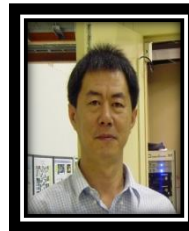
The simulations of continuous model by using commercial COMSOL Multiphysics 3.4 software are presented in the form of ECs migration graphs and as a series of ECs clusters number images for better visualization, originally proposed by Anderson and Chaplain (1998). At the same time, the effects of a series of initial ECs clusters number releasing from parent vessel on ECs density distribution are also addressed. Meanwhile, the use of MATLAB in discrete model illustrates the important role of technology in research in angiogenesis modeling. It is capable of capturing the movements of individual sprouts and producing the morphology of capillary networks observed in vivo. The results show qualitative resemblance to experimental vascular networks, such as this shown in Asahara et al. (1998).

This is a first step towards emulating real experiments through a hybrid model, there are still a considerable number of more individual factors that can be incorporated to understand mechanisms of interactions among different factors during angiogenesis and generate experimentally testable hypotheses. This will be pursued in future work.

REFERENCES

- [1] J.C. Bowersox & N. Sogente (1982), “Chemotaxis of Aortic Endothelial Cells in Response to Fibronectin”, *Cancer Research*, Vol. 42, Pp. 2547–2551.
- [2] J. Folkman & M. Klagsbrun (1987), “Angiogenic Factors”, *Science*, Vol. 235, Pp. 442–447.

- [3] C.L. Stokes & D.A. Lauffenburger (1991), "Analysis of the Roles of Microvessel Endothelial Cell Random Motility and Chemotaxis in Angiogenesis", *Journal of Theoretical Biology*, Vol. 152, Pp. 377–403.
- [4] J. Folkman (1995), "Angiogenesis in Cancer, Vascular, Rheumatoid and other Disease", *Nature Medicine*, Vol. 1, Pp. 21-31.
- [5] M.A.J. Chaplain (1996), "Avascular Growth, Angiogenesis and Vascular Growth in Solid Tumors: The Mathematical Modeling of the Stages of Tumor Development", *Mathematical and Computer Modeling*, Vol. 23, Pp. 47–87.
- [6] W. Risau (1997), "Mechanisms of Angiogenesis", *Nature*, Vol. 386, Pp. 671-674.
- [7] A.R.A. Anderson & M.A.J. Chaplain (1998), "Continuous and Discrete Mathematical Models of Tumor-Induced Angiogenesis", *Bulletin of Mathematical Biology*, Vol. 60, Pp. 857–900.
- [8] T. Asahara, D. Chen, T. Takahashi, K. Fujikawa, M. Kearney, M. Magner, G. Yancopoulos & J. Isner (1998), "Tie2 Receptor Ligands, Angiopoietin-1 and Angiopoietin-2, Modulate VEGF-Induced Postnatal Neovascularization", *Circ. Res.*, Vol. 83, No. 3, Pp. 233–240.
- [9] B.D. Sleeman & I.P. Wallis (2002), "Tumor Induced Angiogenesis as a Reinforced Random Walk: Modeling Capillary Network Formation without Endothelial Cell Proliferation", *Mathematical and Computer Modeling*, Vol. 36, Pp. 339–358.
- [10] S. Sun, Mary F. Wheeler, M. Obeyesekere & Charles W. Patrick Jr. (2005), "A Deterministic Model of Growth Factor-Induced Angiogenesis", *Bulletin of Mathematical Biology*, Vol. 67, Pp. 313–337.
- [11] N.M. Pandya, N.S. Dhalla & D.D. Santani (2006), "Review: Angiogenesis-a New Target for Future Therapy", *Vascular Pharmacology*, Vol. 44, Pp. 265-274.
- [12] B. Addison-Smith, D.L.S. McElwain & P.K. Maini (2008), "A Simple Mechanistic Model of Sprout Spacing in Tumour-Associated Angiogenesis", *Journal of Theoretical Biology*, Vol. 250, Pp. 1–15.
- [13] Michael L.H. Wong, Amy Prawira, Andrew H. Kaye, Christopher & M. Hovens (2009), "Review-Tumor Angiogenesis: Its Mechanism and Therapeutic Implications", *Journal of Clinical Neuroscience*, Vol. 16, Pp. 1119–1130.
- [14] F. Billy, B. Ribba, O. Saut, H. Morre-Trouilhet, T. Colin, D. Bresch, J.P. Boisse, E. Grenier & J.P. Flandrois (2009), "A Pharmacologically Based Multiscale Mathematical Model of Angiogenesis and its Use in Investigating the Efficacy of a New Cancer Treatment Strategy", *Journal of Theoretical Biology*, Vol. 260, Pp. 545–562.
- [15] Y. Cai, S. Xu, J. Wu & Q. Long (2011), "Coupled Modelling of Tumour Angiogenesis, Tumour Growth and Blood Perfusion", *Journal of Theoretical Biology*, Vol. 279, Pp. 90–101.
- [16] T.H. Tsai (2013), "Numerical Investigations of Angiogenesis Induced by Tumor", *International Congress on Chemical, Biological and Environmental Sciences (ICCBES)*, Grand Hotel, Taipei, Taiwan.



Tsung-Hsun Tsai earned the M.S. and Ph.D. degrees in mechanical engineering from National Chung Hsing University, Taichung, Taiwan, in 1996 and 2004, respectively. He is currently with the Department of Mechanical Engineering, WuFeng University, Chiayi, Taiwan, as an Associate Professor. His research interests are in LIGA, microfluid, multilayer porous media flow and biomedical simulation, etc. He has some publications in the areas of HAR electroforming technology for the LIGA process, nanofluid-cooled microchannel heat sink and porous media flow.