Deterministic Simulation of Growth Factor-Induced Angiogenesis

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1. Introduction

Angiogenesis is the formation of new vessels from existing vasculature. The development and growth of new tissue *in vivo* requires adequate transport of nutrients to the tissue and removal of waste products. It is well known that vascular patterning and assembly plays a predominant role in controlling hemodynamic coupling and mass transport [1]. As molecular diffusion has an influence range of only 1-2 mm, angiogenesis dedicates tissue growth and the fate of tissue survival. As a result, angiogenesis is an important cellular process for numerous clinical indications in the adult, including ischemia reperfusion, myocardial and cerebral insufficiencies, wound healing, tissue regeneration, and cancer. For example, inhibition of angiogenesis is a major clinical goal for the undesired tissue growth in cancerous tumors. In order to grow and metastasize, tumors must be able to promote blood vessel growth from nearby capillaries, and switch to an angiogenic phenotype. For any reparative strategy, it is critical to understand the formation and structure of a capillary network.

For several decades, investigators have been attempting to model the morphology of capillary networks. Early models were constrained to one spatial dimension for the simplicity of modeling and the availability of analytical solutions [2-5]. These models provided useful information of angiogenesis such as average sprout density and network expansion rates, and some had valuable medical applications. However, the actual structure and morphology of the capillary network is unattainable by these models due to their one-dimensional framework. More realistic continuum models of angiogenesis in two spatial dimensions [6-7] provided a more detailed information for the spatiotemporal distribution of capillary sprouts, but were still not possible to incorporate certain important events such as repeated sprout branching and hence could not provide the overall dendritic structure of the capillary network. Stokes and colleagues [8-9] proposed a probabilistic model in two spatial dimensions based on stochastic differential equations, where the motion of individual endothelial cells was described. Random motility, chemotaxis, sprout branching and anastomosis were incorporated into the mathematical system, but this model was not able to describe the interactions between the endothelial cells and the extracellular matrix (ECM). Chaplain and colleagues [10-11] developed a discrete *in silico* model describing the formation of a capillary network via endothelial cell migratory and proliferative responses to external chemical stimuli and ECM. However, the group used a discrete morphological model to simulate blood flow through capillary networks around solid tumors rather than a continuous model. Similarly, Levine *et al.* [12] have developed a model to simulate tumor vascularization based on biochemistry at the cell level, random walk motility of endothelial cells, and standard transport equations for the diffusion of bioactive factors through ECM. However, this model simulates only propagation fronts of growth factors and angiogenesis, but not vascular pattern formation. In this paper, we implement a continuous and deterministic approach for tissue angiogenesis [13], and report preliminary results on its application to the simulation of a tumor-induced angiogenesis.

2. Mathematical Model

In our deterministic 3D (x, y, t) angiogenesis model, capillary presence is represented by an indicator function, n. This indicator function is a function of space (x, y) and time t, and possesses the binary value of either 0 or 1 depending on the presence of capillaries [13]. We submit that a binary value indicator function is a more useful descriptor of capillary network morphology at a fine scale and captures the network information more precisely than traditional capillary density functions. The concentration of chemotactic growth factors (CGF) is denoted by c_{CGF}. The fibronectin concentration, denoted by c_{FN}, is employed to represent a single component of ECM. The system of governing equations for these three variables is:

$$n = F(\bar{S}) \tag{1}$$

$$\frac{\partial c_{CGF}}{\partial t} = \nabla \cdot (\mathbf{D} \nabla c_{CGF}) + \alpha_{CGF}(1-n) - \lambda_{CGF} nc - \lambda^*_{CGF} c_{CGF} \tag{2}$$

$$\frac{\partial c_{FN}}{\partial t} = \omega_{FN} n - \mu_{FN} nc_{FN} \tag{3}$$

The capillary indicator function, n, at any given time t is assigned to be 1 over the set $\overline{S}$ that describes the position of all sprouts. The partial differential equation (2) for CGF concentration is obtained from a mass balance incorporating accumulation over time, diffusion over space, production by ECM, consumption by endothelial cells and loss by natural decay. Fibronectin is known to be attached to ECM and does not diffuse, and the equation (3) for c_{FN} contains only three terms, representing accumulation, production and the uptake of fibronectin by endothelial cells. The detailed dynamics affecting the function of n are modeled by the following equations:

$$\mathbf{u} = \mathbf{K}(k_c \nabla c_{CGF} + k_f \nabla c_{FN}) \tag{4}$$

$$\frac{d\mathbf{p}}{dt} = k_p \frac{\mathbf{u}}{|\mathbf{u}|}, \ \mathbf{p} = (x_i, y_i), \ \forall (x_i, y_i) \in S \tag{5}$$

$$S(t^+) = A\left(B\left(S(t^-)\right)\right) \tag{6}$$

The direction of sprout tip extension and the velocity of sprout tip extension are modeled via equations (4) and (5). Biologically, cells behind the sprout tips undergo mitosis and sprout extension occurs subsequently. The change in position of an individual sprout tip during sprout extension depends upon its direction and its speed. Equations (4) and (5) imitate that endothelial cells respond chemotactically to gradients of CGF and behave haptotactically to gradients of fibronectin in ECM. The tensor **K** in equation (4) is the conductivity of ECM for the movement and extension of capillary endothelial cells. Biologically, capillaries cannot form without the presence of ECM. **K** is an intrinsic property of ECM and is permitted to be heterogeneous and anisotropic. ECM is naturally heterogeneous. The anisotropy of ECM means that the resistance of ECM for the sprout extension may be strong in some directions and weak in others. This is realistic since natural ECM can possess structured orientation (e.g. layers). The anisotropy of ECM implies that the direction of sprout extension does not necessarily coincide with the direction of CGF concentration gradient. The speed parameter, k_p, in equation (5) is a function of CGF concentration, and is further modeled by a cell-cycling model incorporating the endothelial cell length and the dynamic cell cycle time [13]. The operators A and B in equation (6) represent vessel anastomosis and sprout branching, respectively. Operator A models the fusion of blood vessels including the tip-to-tip and the tip-to-sprout anastomosis. Operator B describes the generation of new sprouts from existing sprout tips when the age of the current sprout and the variation of the normalized velocity vector satisfy certain conditions [13].

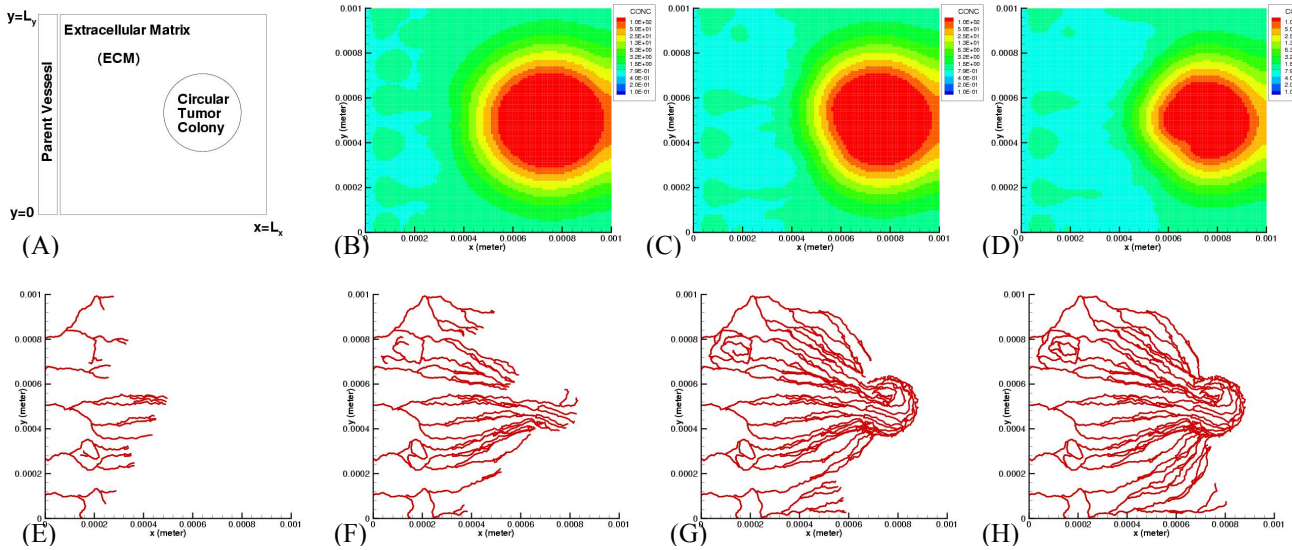

Figure 1: Simulation of a tumor-induced angiogenesis. (A) Domain geometry; simulated CGFs concentration profiles at (B) 100, (C) 150, and (D) 200 time steps; and simulated capillary network at (E) 100 (F) 150, (G) 200 and (H) 250 time steps.

3. Results

We apply the angiogenesis model to simulate a tumor-induced angiogenesis using the implicit cell center finite difference-based algorithm [13, 14]. The simulation geometry is illustrated in Figure 1(A). The value of α_{CGF} in the tumor area is 6.42×10^{-18} mol/(m^{2}s), while it has a value of $\alpha_{CGF}=6.42\times10^{-21}$ in the remaining extracellular matrix. A uniform time step of 0.14 days is employed for time integration. The threshold age for branching is 3 days. The initial tips are automatically generated at a certain position of the left boundary where maximum concentration is achieved. One tip is generated for every 10 time steps starting from the beginning of simulation up to 50 time steps, which results in 5 starting tips generated from the parent vessel. All remaining parameters are the same as in [13]. Figure 1 illustrates simulations of CGF concentration profiles and capillary network formation as time increases. Clearly, endothelial at the capillary sprout tips are migrating from the parent vessel to the right induced by CGF concentration gradient. The CGF is released from both the extracellular matrix and the tumor cells, but substantially dominated by the latter. The simulation produces capillary networks with realistic structures and morphologies. At the initial stages of angiogenesis where the capillaries are far from the tumor, all capillaries extend to the right, with the central one proliferating fastest among all. At later times, the capillaries converge into the tumor more quickly. This is biologically meaningful because, when sprouts are near the tumor colony, they have a better chance to receive a clear message sent from tumor cells, thus proliferate more frequently and more directly towards the tumor colony.

4. Discussion

Endothelial cells, CGF, ECM, and their coupled interactions are considered in this model. In addition, major mechanisms are incorporated directly into the continuous level, including endothelial cell proliferation, sprout branching, and vessel anastomosis. An advantage of this model is that the complex physical, chemical and biological processes in angiogenesis can be described and consequently analyzed by a continuous mathematical system with self-contained information. This also allows us to apply various numerical algorithms and to select the most efficient schemes to discretize the continuous model without being constrained in the incorporation of additional physical or biological information to the discretized level. The model introduces a new concept, a capillary indicator function, to describe capillary network structure. The replacement of traditional endothelial cell density by the capillary indicator function empowers our model to capture the capillary network sharply in fine scales. We also introduce the conductivity of ECM for the progression of capillary sprouts, another new concept, to describe the heterogeneity and anisotropy of ECM. The application of the model to a tumor-induced angiogenesis demonstrates that the model sharply captures the overall dendritic structure and pattern of the capillary network.

5. References

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