Physical Models of Blood Vessel Formation

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Understanding the mechanism of blood vessel formation has been an important subject in recent medical research. Various experiments and models have been proposed, yet fully understanding the mechanism has been challenging. This paper is meant to be a brief description of experimental results and methods of modeling the blood vessel formation. Models are compared with experimental data for the process of both vasculogenesis and angiogenesis to discuss how physical assumptions in the models lead to the understanding of biological mechanism.

1 INTRODUCTION [1]

Biological viewpoint on blood vessel formation

The human body has a complex circulatory system. Blood circulates in the entire body through arteries, capillaries, the heart and veins. It is these blood vessels that enable efficient exchange of oxygen and nutrients and the removal of waste products in the body. For this reason, blood vessels play a role in virtually every medical condition. Understanding the blood-vascular system and its formation is an important task, because it will directly lead to the cure of various diseases.

Formation of blood vessels begins in the early stage of embryonic development, and is achieved by two successive processes: **vasculogenesis** and **angiogenesis**. Blood vessel formation originates in an aggregations of cells called blood islands that form in the embryonic yolk sac, a membranous sac attached to an embryo. At early stages, a blood island contains a homogeneous collection of cells arising from migrating mesodermal cells. These cells, called hemangioblasts, are the precursor of both blood and blood vessels. As they develop, two types of cells form: hematopoietic stem cells (HSCs) and endothelial progenitor cells (EPCs). As blood islands merge, HSCs, which sit at the center of the blood island, form into blood cells, while EPCs, which sit at periphery of blood island form into a vascular network that grows toward the embryo (Figure 1).

Vasculogenesis is the first stage of blood vessel formation, during which EPCs form lumens, a tube-like structure. In this process, the primary vascular network and meshes of homogeneously sized capillaries are formed. These give rise to the heart and the first primitive vascular plexus inside the embryo and in its surrounding membranes as the yolk sac circulation.

The primitive network formed from vasculogenesis is developed by **angiogenesis**, a process of deformation of pre-existing vessels by sprouting, bridging, and branching. Angiogenesis remodels the primary vascular network into a highly branched hierarchical vascular tree, composed of arteries and veins. Angiogenesis also occurs in adult tissues. In addition, it is the process that occurs in tumor cell development, which will be discussed later, as well as in wound healing.

Linking biology to physics

Since it is such an important task to understand blood vessel formation, research has been done in various fields such as biology, chemistry, physics, and mathematics. However, the process of endothelial cells self-organizing into a capillary network is still not well understood. This is because the process involves many kinds of chemicals (growth factors) and molecular players. As physicists, we can simulate this

[1] This section follows [1], [2], and [3]

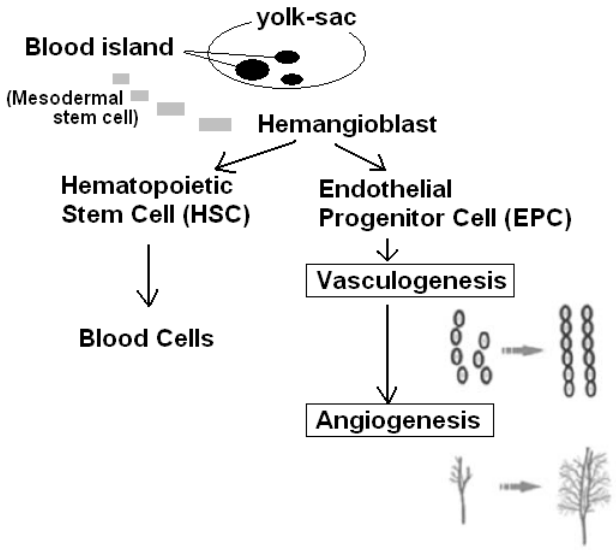

Figure 1: Chart of vascular development

process with various models drawn from fluid dynamics, statistical mechanics, and mathematical analysis like linear stability analysis. In the rest of this paper, some models that use these physical concepts are introduced. Comparing the results to experimental data will show the accuracy of the assumptions of the models. In this way, we can determine the dominant factors that cause the formation of the vascular system, and thereby improve our understanding to the process of vasculogenesis and angiogenesis.

2 VASCULOGENESIS [2]

2.1 Experimental Facts

Vasculogenesis can occur *in vitro*[3] using various experimental set-ups. Therefore, the results vary and this makes it is hard to gain a unified illustration of the actual biological process. In Serini et al. (2003), human endothelial cells are placed on the surface of Matrigel, a gel-like layer with characteristics similar to living tissues. These cells then form a pattern in 12-15 hours as follows:

1. In the first 3-6 hours, endothelial cells migrate independently until they collide with another cell. (Figure 2a-b) During this migration, cells exchange signals

[2]This section follows references [4], [5], and [6]

[3]The technique of performing a given experiment in a controlled environment outside of a living organism

using Vascular Endothelial Growth Factor (VEGF-A), which induce a gradient of migration direction.

2. Cells form a continuous multicellular network. (Figure 2c-d)

3. The formed network moves as a whole, driven by stress fields.

4. Cells fold up to form the lumen of the capillary. Mean chord length is approximately constant at $l \approx 200 \pm 20\mu m$ over the initial cell density of $n_o = 100$ to 200 $cells/mm^2$ (Figure 7a).

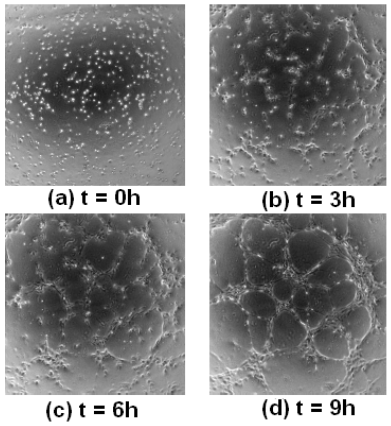

Figure 2: Formation of vascular networks *in vitro*

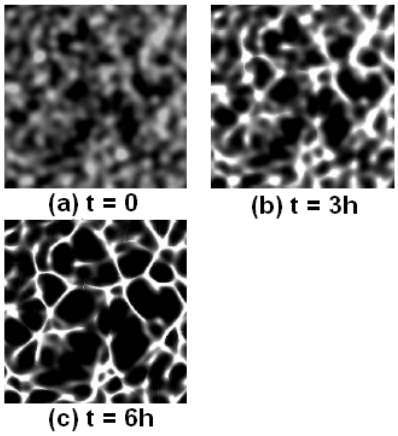

Figure 3: Formation of vascular networks by simulation (Black areas represent regions filled with cells)

The experimentalists also found that the network formation has the following characteristics:

(a) Extinguishing VEGF-A gradients results in strong inhibition of network formation. VEGF-A plays a role in defining the mesh size. (Figure 4)

(b) Formation of a coarser net would cause necrosis of the tissues, and the formation of finer net will be useless. Therefore forming appropriate chord length is important.

(c) Vascular networks fail to develop outside of the initial density range n_o. Single connected network breaks down in groups under critical density of $n_o \approx 100$ $cells/mm^2$. This is a percolative transition (Figure 5).

Figure 4: Change in mesh size in response to abundance of VEGF *in vitro*

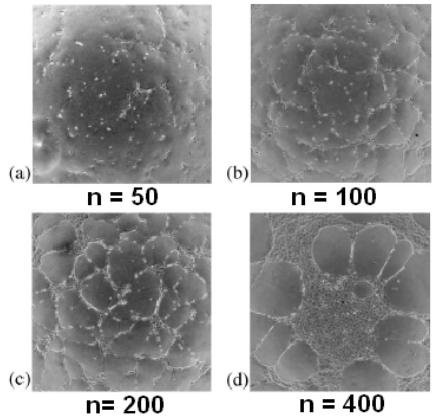

Figure 5: Dependence on the initial cell density, *in vitro* (in the unit of [cells/mm^2])

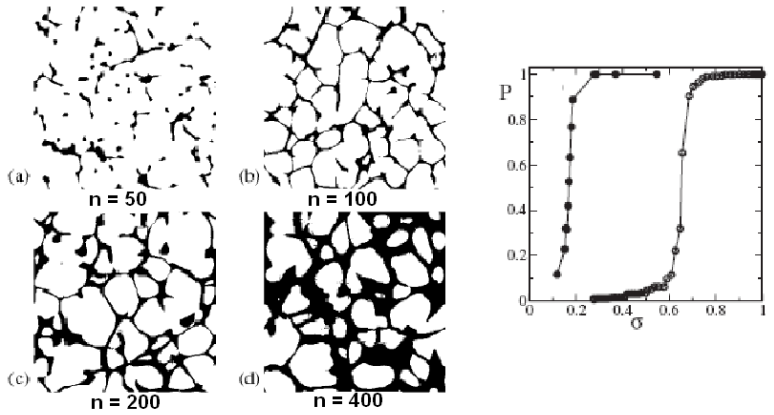

Figure 6: (left) Dependence on the initial cell density, numerical simulation(in the unit of [cells/mm^2]) (right) Percolative transition of vascular network

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2.2 Models and simulations

Two complementary models have been established to describe the process of vasculogenesis. These are the persistence and endogenous chemotaxis model (PEC model) and the elasto-mechanical model. The PEC model is used to describe early vasculogenesis where uniform cells migrate to form a two-dimensional primitive vascular system. The elasto-mechanical model, on the other hand, describes the formation of three-dimensional lactunae (capillaries) starting from this monolayer initial conditions.

PEC model

The PEC model assumes that the size of the network is related to the product of the diffusion constant and the half-life of the chemical factor, VEGF-A. This assumption is deduced from 2.1.1 and 2.1.(a). This model can be described by the following equations from Gamba *et al.*[5], where n is the density of endothelial cells, $\mathbf{v}$ is the velocity of endothelial cells, and c is the density of chemoattractant:

$$\frac{\partial n}{\partial t} + \nabla \cdot (n\mathbf{v}) = 0 \qquad \text{(continuity equation)} \tag{1}$$

$$\frac{\partial c}{\partial t} = D\nabla^2 c + \alpha n - \frac{1}{\tau} c \qquad \text{(diffusion equation)} \tag{2}$$

$$\frac{\partial (n\mathbf{v})}{\partial t} + \nabla \cdot (n\mathbf{v} \times \mathbf{v}) = \mathbf{f} \qquad \text{(Riemann equation)} \tag{3}$$

D, α, τ, are diffusion coefficient, the rate of release, and characteristic degradation of cell response, and $\mathbf{f}$ models the cell persistence[4]. Using these equations, cells are modeled as a fluid accelerated by gradients of the soluble factor. Initial conditions are a set of randomly distributed bell-shaped bumps in the density field, representing the spread of single cells with zero velocity.

The results of simulation (Figure 3), show a pattern that resembles that of experiment *in vitro* (Figure 2). The general features of the patterns are independent of initial conditions, and they have a mean chord length of $l \approx 100-200\mu m$, which agrees with 2.1.1. The result also shows that by varying the initial cell density, percolative transition occurs (compare Figure 5&6(left)) below $n_c \approx 100$ cells/mm^2, which agrees with 2.1.(c). Figure 6 (right) shows this transition studied by Szabo *et al.* [7] using a similar model. σ represents the volume fraction; the line with open symbol is simulated with initial condition of overlapping disks; and the line with solid symbols is simulated with non-overlapping disks. After the transition occurs, connected network can no longer form.

Elasto-mechanical model

The elasto-mechanical model is based on the idea that mechanical forces play a major role in capillary formation as in 2.1.3. It assumes that cells exert traction forces onto the extra-cellular matrix (ECM), the extracellular part of animal tissue that usually provides structural support to the animal cells, which also experience a drag force by the Petri dish. This model, which varies in different papers, can be summarized by the following rather general equations:

$$\frac{\partial n}{\partial t} + \nabla \cdot \mathbf{J} = \Gamma \qquad \text{(mass equation 1)} \tag{4}$$

$$\frac{\partial \rho}{\partial t} + \nabla \cdot \left(\rho \frac{d\mathbf{u}}{dt} \right) = -\Delta \qquad \text{(mass equation 2)} \tag{5}$$

$$-\nabla \cdot \mathbf{T}_n - \nabla \cdot \mathbf{T}_\rho = \mathbf{F}, \qquad \text{(force balance equation for cell \& Mitrigel)} \tag{6}$$

where n is the density of endothelial cells, ρ is the density of ECM, and $\mathbf{u}$ is the displacement of extracellular matrix from its original position. $\mathbf{J}$, Γ, Δ, $\mathbf{T}_n$, $\mathbf{T}_\rho$ are

[4] $\mathbf{f}$ is a combination of different factors. Refer to [4] for details

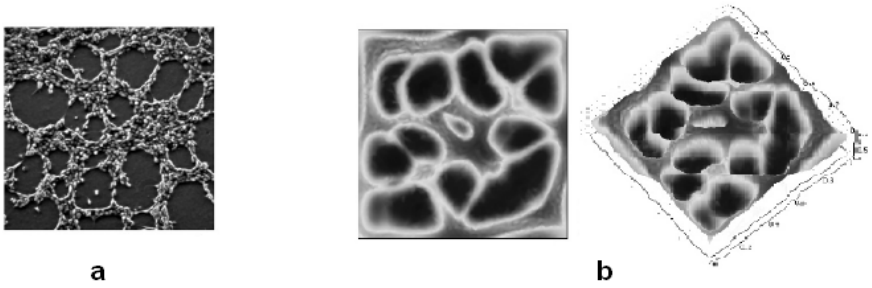

Figure 7: Formation of capillary-like networks for (a)experimental observation, and (b)3D simulations

cellular flux, generation and death of cells, digestion of ECM by the cells, cell traction stress, and stress in deformed ECM. **F** is drag or restoring force between ECM and Petri dish. Instead of uniformly distributed cells, a monolayer network like the result of the simulation of the PEC model is used as initial conditions.

Linear stability analysis performed in a neighborhood of the homogeneous steady state of these formulae shows the existence of a critical density n_o, under which instability occurs. This leads to the critical cell traction stress T_c, above which the pattern formation occurs. This result suggests that it is necessary to have a sufficiently high number of cells to trigger the formation of patterns. Otherwise, the uniform distribution is stable.

Simulations of this model beginning with a region with fewer cells surrounded by more cells, shows that as the region with high cell density thins, lacunae of about 300-500 μm form surrounding the region with lower cell density (Figure 7b). This agrees well with the experimental result (Figure 7a). The simulation also shows that the shear and bulk viscosity strongly affect the size of formed lacunae. The evolution of the cells is dynamic so that patterns do not reach a steady state, in contrast to the PEC model. As time passes, lacunae may enlarge and decrease in numbers.

2.3 Discussion & Analysis

The PEC and the elasto-mechanical models indicate that persistence and chemotaxis are associated with migration of cells, while mechanical interactions are associated with formation of capillaries.

The process of vasculogenesis based on these two models can be physically analyzed in the following way. An initially random but locally inhomogeneous cell density distribution causes an inhomogeneous chemical gradient to diffuse in the system. Cells migrate according to this chemical gradient, and form regions of thick and thin cells. A non-linear dynamical mechanism in sharpens the ridges and empties the valleys so that the cells will form a monolayer network with characteristic chord length $\approx$

l. (PEC model) When cells reach sufficient density, they feel enough traction stress to trigger capillary formation. Cell traction stress overcomes the restoring force of ECM and attraction force of the dish. Finally, cells start to attract each other to form lacunae. (elasto-mechanical model)

The two models seem to suggest that cells can change their motion depending on the environment. That is, depending on their initial distribution, cells can either migrate or form capillaries by attraction. Therefore, it will be worthwhile to suggest a universal model that connects the migration and pulling regime of cell movement. It will also be useful to perform simulations with more parameters in the equations to account for other chemicals. Finally, in Serini et al. (2003), the experiment is performed using only endothelial cells. These cells, *in vivo*, form outer layers of the blood island, surrounding blood cells. Capillaries form by merging of these blood islands. Observing how closely this experiment follows the real blood vessel formation and its simulation will contribute to further understanding of blood vessel formation.

3 ANGIOGENESIS[5]

Angiogenesis, the process of growth and remodeling of the primitive network, occurs in both the embryo and the adult body. However, the molecular basis of angiogenesis in the embryo is different from that of pathological angiogenesis in the adult. For example, nitric oxide, which plays an important role in adult angiogenesis, is not necessary for embryonic angiogenesis. The focus of embryonic angiogenesis is in the formation of tree-like structures. On the other hand, adult vasculogenesis is often disease induced or associated with wound healing. In this section, I will show that a simple physical model can reproduce embryonic angiogenesis, and describe directional angiogenesis in adult body using a model for tumor-induced angiogenesis.

3.1 Embryonic angiogenesis

3.1.1 Experimental Observation

From the *in vivo* research on chicken embryos, researchers have found that there are three phases of angiogenesis: sprouting, intussusceptive microvascular growth, and simple expansion. Blood flow is introduced during these process, and contributes to the directionality of branches. Capillary networks that consists of many closed circuits will change to a bifurcating structure with a flow. This is difficult to study *in vitro* because we cannot realistically introduce a flow into a capillary network. In chicken embryos, a branching network is developed in two dimensions in a membrane. The studies *in situ*[6] as in Figure 8 show that tree-like blood vessels in chicken embryos

[5]This section follows references [8], [10], and [9]

[6]experiment carried out exactly in place where it occurs.

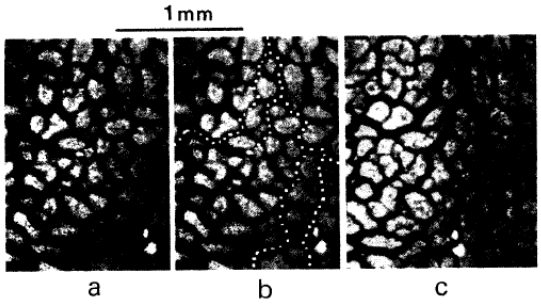

Figure 8: Angiogenesis in chicken embryo. (a)polygonal capillaries (b)&(c)formation of branches

form from polygonal patterns. Vessels in a network, indicated by the dots, thicken and form branches along which blood flows continuously.

3.1.2 Models and Analysis

H. Honda *et al.* [9] show by computer simulation that the transformation of the vascular pattern from the capillary network to the branching structure is a self-organizing process. Starting from polygonal patterns, branch formation is simulated using positive feedback process. Their assumptions are

1. Vascular systems can be simulated as a electrical circuit system. The heart, arbitrarily positioned, is a current source and blood vessels have resistance.

2. Resistance of each vessel section is variable. Thicker vessels have less resistance, while thinner vessels have more resistance.

3. Above and below threshold resistances R_{max} and R_{min}, the resistance will be infinite and zero respectively. This means that a blood vessel will vanish if its resistance becomes too high.

Figure 9 shows their computer simulation of blood vessels under these assumptions. They discovered that the transformation of capillary network to the branching structure takes place even if the initial capillaries are homogeneous in size. They argue that this process repeats during the growth of an embryonic body, and that is why the vascular system develops adaptively with the growth of the individual body.

Actual capillaries have variations in size, sprouting, and branching. Simulations accounting for these factors would be a useful investigation. The symmetry breaking in position and direction of sprouting and branching should also be investigated.

3.2 Adult angiogenesis

3.2.1 Experimental Observation

A tumor has the ability to trigger the formation of a vascular network. The Tumor Angiogenic Factors(TAF), will induce a chemical gradient in the system, and therefore blood vessels migrate by looping and branching toward the tumor. Specifically, in response to growth factors like TAF, the membrane of blood vessels undergoes degradation. As a result, vascular endothelial cells (ECs) can migrate and induce the formation of an intermediate, new membrane of blood vessel.

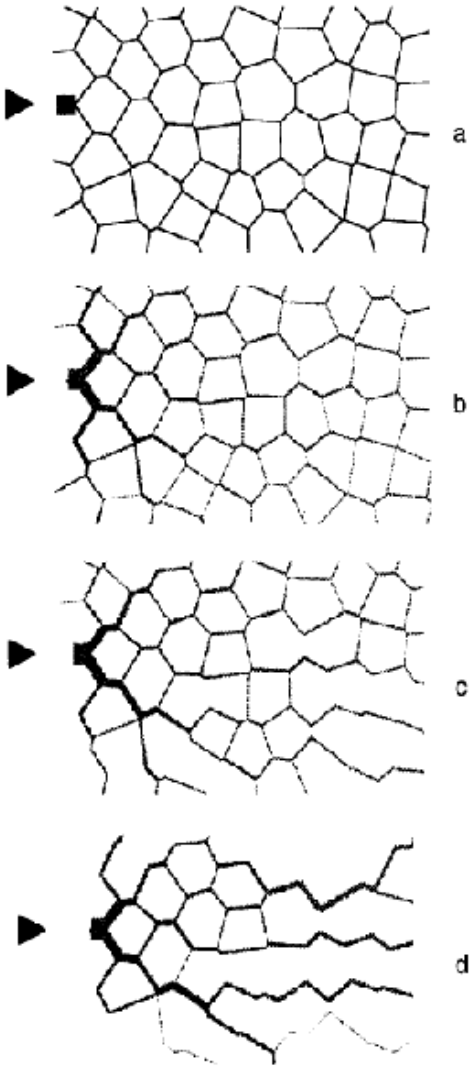

Figure 9: Simulation of angiogenesis in embryo ((a)-(d) in the oder of time steps, triangle indicates the position of heart)

3.2.2 Models and Analysis

Chaplain and Anderson [11], and M. Pindera[10] use reinforced random walk, an approach based on the notion that cells undergo quasi-random migration with a preferred direction, to simulate tumor angiogenesis. They used the following equation,

$$\frac{\partial \eta}{\partial t} = D\nabla\cdot\left(\eta\nabla\left(ln\frac{\eta}{\tau}\right)\right) \quad \text{(diffusion-like equation)} \tag{7}$$

where η is EC concentration, D is the diffusion coefficient and τ represents a transition probability of the cell propagating direction, along with an equation of chemical drift velocity induced by TAF. The simulation is performed in two steps: (1) Initiation of new branch sites for vessel branching and formation, and (2) Migration toward the tumor. Figure 10 shows the result of this simulation, in two dimensions, which agrees with dimensional and transitional characteristics in experiment.

The simulation shows that the density of ECs necessary to allow capillary branching is inversely proportional to the distance from the tumor and proportional to the concentration of TAF. This result agrees with experimental observation that the distance between successive branches along the capillaries decreases as they get closer to a tumor.

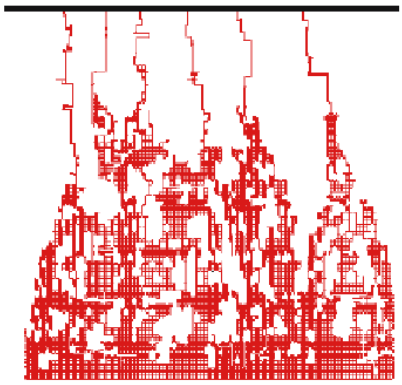

Figure 10: A typical simulation result of tumor angiogenesis

4 Conclusion

In this paper, various models of a vascular system were discussed. Some of the results contribute to findng the parameters that play the major role. Others give a coarse grained simulation of vascular network formation. These are some examples of how physics can contribute toward the understanding of biological system. Of course, these simulations are not complete. They overlook factors like the collapse of a newly formed vessel, adhesive properties of ECs, inhibitors, diffusion of a drag, etc. By refining our model, we can expect to further our understanding of these systems.

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