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DIFFUSION LIMITED AGGREGATION FROM SHEAR STRESS AS A SIMPLE MODEL OF VASCULOGENESIS

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Abstract

A model is proposed by which the formation of the vascular network in animals proceeds via progressive penetration of the vessel ramification into a capillary mesh, by means of a laplacian growth mechanism of hydrodynamical origin. In this model, the growth of both arteries and veins follows the directions of high shear stress provoked by the blood flow on the endothelial wall of a pre-existing capillary mesh. This process is shown to be identical to the phenomenon of dendritic growth, which is responsible for the formation of such well-known patterns as dendritic crystals, lightning sparks or branching aggregates of bacteria. A number of straightforward consequences of potentially important medical and physiological interests are deduced. These include the natural and spontaneous organization of the arterial and venal trees, the spontaneous and unavoidable tropism of arteries towards veins and *vice-versa*, the hierarchical character of the vessels and the possibility of computerized prediction of the vascular pattern from the shape of the capillary bed.

1. INTRODUCTION

Branching patterns are ubiquitous in nature. In the context of out-of-equilibrium self-organized morphogenesis, branching patterns known as *fractal dendrites* have been studied by physicists.[1] These include fractal crystals, dielectric breakdown patterns, viscous fingering, etc.[2,3] These trees display spontaneously a fractal hierarchical structure. Their growth is driven by a field satisfying the laplace equation around the tree, which imposes a growth velocity of the tree surface proportional to

the gradient of the field (the higher the flux, the higher the growth speed). It appears from simple inspection that these physical patterns possess some kinship with vascular trees: ease of formation, self-organization, non-deterministic structure, hierarchical branching and no loops. This has been pointed out by both physicists[4] and physicians,[5] but the lack of a mechanism of vasculogenesis has so far hindered any significant progress towards that unification (reviewed in Ref. 6). We will propose in this paper that vasculogenesis indeed belongs to the dendritic class of patterns, by means of a laplacian growth process driven by pressure in the capillary bed.

During embryonic morphogenesis, a functioning cardiovascular system is required to support the metabolic demands of the growing embryo. As a consequence, vasculogenesis occurs rather early in the growth process. Two mechanisms of vascular growth are observed. The first is the local transformation of cells into fibroblasts, endothelial cells etc. which leads to random formation of capillary segments that eventually percolate. The second is the proliferation and migration of endothelial cells found in the first vascular structures which leads to sprouting into previously avascular organs. In both instances, remodeling of the initially homogeneous capillary network, leads to the formation of small vessels,[7,8] that will subsequently enlarge in a process of maturation called pruning. This process of maturation of a set of small vessels into a pattern that resembles a tree is far from understood and as explained in a very recent biological review by Risau,[9] "its molecular mechanisms remain obscure." This remodeling leads to a branching structure of vessels into primary, secondary, etc. vessels. Vasculogenesis occurs only during early embryogenesis whereas angiogenesis is required for the normal growth of both the embryonic and postnatal tissues and in pathological situations such as wound healing, tumor growth, natural or artificial anastomosis, etc.[7,10,11] Let us however, remember that the actual existence of a beating heart is not compulsory for vascularization; indeed, in many small invertebrates, circulation of the blood is provided by the contraction of the entire body, as it is partially in vertebrates (most notably in the intestine). A beating heart is only an adaptation of a specific muscle to pumping.

In a vessel, the endothelium is located between the blood flood and the vascular wall itself. It is well-established that cells lining the blood circulation are exposed to strong fluid forces, especially viscous shear. Consequently, mechanically related responses controlled by the endothelium have evolved as part of normal vascular physiology. The response can be passive (simple mechanical deformations) or active (chemically-mediated) and the transmission of hemodynamic information from the blood to the underlying vessel wall originates in the endothelium. Let us remark that, indeed, the only place where the information about the flux in a tube can be transmitted to the living tissue, is at the tube surface and the very fact that the vasculature of vegetals and animals has a varying diameter is an indication that receptors of flux exist on the vascular walls. Hence, as reviewed in Ref. 12, hemodynamic factors (defined as mechanical forces in the flowing blood) influence endothelial biology either by the direct action of shear stress and stretch forces on the endothelium itself, or by indirect modification of the local concentrations of chemical agonists at the endothelial surface. These mechanisms are not mutually exclusive. In the end, endothelial cells might respond to shear flow in order to satisfy a specific mechanical constraint, which is under genetic control and which, in some instances, has been quantitatively estimated.[12]

That vasculogenesis is affected by flow has been hypothesized for more than a century.[13,14] The establishment of the vascular pattern was proven to depend on fluid circulation long ago. In 1972, Flaherty *et al.* demonstrated that endothelial cellular morphology could be changed by flow by resecting an arterial patch at 90° to its original orientation and observing how the endothelium re-aligned with the flow.[15] More recently, it was shown experimentally that endothelial cells respond to shear stress (Refs. 12, 14, 16 and references therein) and that capillaries enlarge under the influence of blood flow during embryogenesis,[8] which confirmed historical data on this question, reviewed in Ref. 13. It remains to be understood how such a response may lead to a hierarchical organization of the vascular tree.

2. THE MODEL OF VESSEL GROWTH ACROSS THE CAPILLARIES

If we now consider the capillary bed, prior to vasculogenesis, as a discretized lattice of small tubes in which blood flows and, introduce the sensitivity

of the endothelial cells to shear, it is easy to construct a physical growth model of vasculogenesis in the following way.

In a capillary tube, the pressure drop between two vertices (two capillary crossroads) is related to the flow V in the middle of the tube that links the two vertices by the Poiseuille law :

$$(R^2/8\eta)\ \text{grad}\ P = -V \tag{1}$$

where R is the radius of the tube and η the viscosity of the blood.

If we consider now the entire lattice of the capillary bed, the pressure at each vertex is obtained, in the simplest hypothesis, by solving the conservation equation for the fluid flow (incompressibility), which is given by

$$\Delta P = 0\,. \tag{2}$$

We next implement the growth of vessels by the following simple rule: wherever the shear is large, the local capillary segment is progressively replaced by a segment of vessel. This change amounts to introducing a speed v of growth of the branched pattern which is proportional to the shear stress. We take as simplifying assumption that the larger vessels are so much larger than the capillaries, that the pressure drop across them is extremely small (stated otherwise, the vein or artery cross-section is much larger than the cross-section of the capillaries). The shear stress σ at the very surface of one small tube is proportional to the gradient of the fluid speed taken at that surface, so it is proportional to the fluid speed V in the middle of the tube. It is also proportional to viscosity (the higher the viscosity, the higher the shear) and to the inverse of the radius of the tube (the larger the tube, the smaller the shear). In the end, the shear stress σ, the fluid speed in the center of the tube and the pressure gradient are related by

$$\sigma = 2(\eta/R)V = (R/4)\ \text{grad}\ P\,. \tag{3}$$

As a consequence, the direction in which the pattern of large vessels is more likely to elongate is the direction of high pressure gradient. The growth speed of the vascular interface through the capillary bed will be proportional, in this simple model, to the gradient of P taken at the border of the tree of large vessels (the so-called harmonic measure in physics)

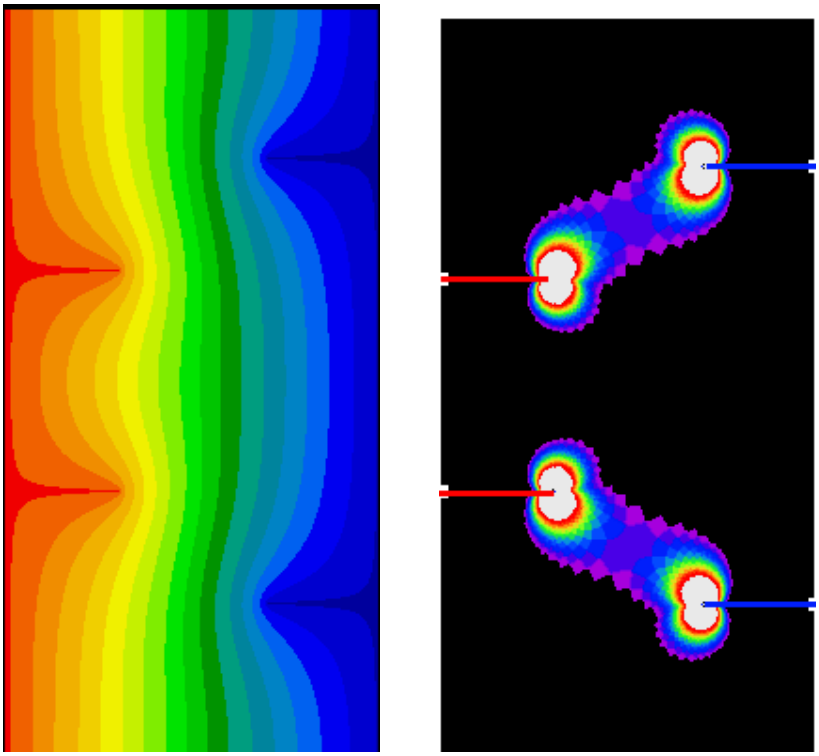
$$v = -k\ \text{grad}\ P \tag{4}$$

where k is a physical, normalizing parameter and v is the local, instantaneous growth speed of the vessel tree (not the fluid speed V). This growth speed varies from place to place.

It is known that transfer of fluid shear stress forces to the cell occurs first at the luminal cell surface. Plasma membrane molecules are therefore candidate mechano-transducers.[16] Mechano-transduction is certainly modulated by changes in the stiffness of the membrane receptors linked to varying lengths of external hydrophilic loops and by the stiffness of the coupling between the receptor and the cytoskeleton itself, coupled to structure and geometry of the tissue. These questions have been extensively reviewed in Refs. 12 and 16. Therefore, the parameter k represents very crudely the time scale of the sequence (membrane transduction)-(cell reaction) until a large vessel is completed. This time scale is much larger than the time scale of the establishment of the pressure drop. Indeed, the time scale for physical change of the vessel is of the order of an hour, while the time scale for the establishment of the pressure pattern is of the order of seconds. In the end, the model in this simplifying version, is identical to the so-called Diffusion Limited Aggregation (DLA) model of growth in its Dielectric Breakdown Model (DBM) version,[1–3] which is well-known to give fractal branching patterns.

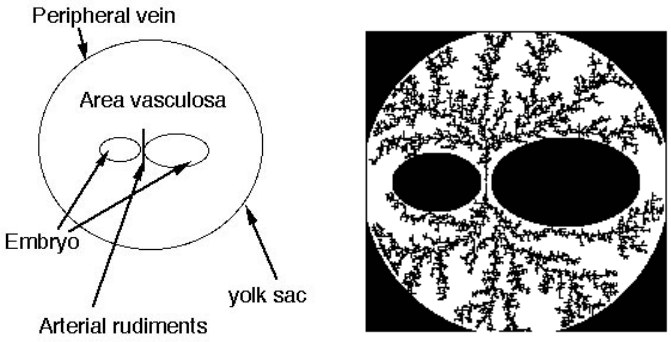
The model presented here catches several essential features of the growth of vascular trees. First of all, arteries and veins will exhibit a spontaneous tropism toward each other. Indeed, if we consider two arterial (red) and two venal (blue) rudiments (Fig. 1) and solve for the laplacian field and for the shear stress in the capillary bed, we see that the shear stress will be higher in a region extending between the neighboring arteries and veins. A vein will spontaneously grow (replacing capillaries) towards the closest artery and *vice-versa*. In Fig. 1(b), the tropism appears very clearly with a threshold color table for the shear. This is the analog of spark propagation between two electrodes in electrostatic problems.

The model also explains or predicts structural properties of vascular trees. As an example, we consider the establishment of the *area vasculosa* outside the chick's embryo, as classically observed through the eggshell (see for example Ref. 8, Fig. 1 or Ref. 13, Fig. 390). It has been shown that a capillary bed establishes on the yolk sac in the first hours of embryogenesis and that a branching pattern is



(a) **(b)**

Fig. 1 **(a)** Numerical calculation of the pressure between two arteries and two veins. The laplace equation is discretized on a lattice, which represents the discretized lattice of small capillaries. The lines of constant P are represented with a color table. Each pixel of this image is a vertex of the capillary bed and the calculation contains 200×400 vertices i.e. 80 000 capillary segments; **(b)** Shows the map of shear stress in the same configuration. This map gives the global tropism of the artery and vein growth through the capillary bed. One observes a spontaneous tropism of arteries towards veins and *vice-versa*, under the influence of the hydrodynamical flow. There is no need of a diffusing morphogenetic effector, the pressure suffices to self-organize the pattern. Moreover, the tropism is symmetrical and independent of scale for length scales larger than the capillary size, because the laplace equation [Eq. (2)] is scale invariant. Scales would enter naturally into the problem if, for example, a finite resistance of the larger vessels was used, instead of an infinite conductivity (as is the case here).



(a) **(b)**

Fig. 2 **(a)** Scheme of the yolk sac and position of the embryo. The embryo is represented by two lobes, one anterior and one posterior lobe. Veins and arteries start at right angles from the center of the embryo; **(b)** DLA simulation in a schematized yolk sac showing the organization of the arterial tree. The random walkers are launched from a circle far away from the heart and they attach one after the other to the growing pattern. The DLA forms a random branching pattern, which is a *Monte-Carlo* solution of the DBM. While some minor technical aspects may be different, the mechanism of growth appears obviously. Branches growing around the lobes are well-reproducible. The white area is not a non-vascularized area, but a lattice of minute capillaries, which are not represented for the sake of clarity.

well-formed by about h. 80 after fertilization.[8] Branching starts when flow establishes i.e. when the capillary bed percolates and the heart beats. The structure of the embryo, as schematized in Fig. 2(a), is composed of two large lobes along an antero-posterior (A-P) polarity, with the heart located between the two lobes. The embryo is at one pole of the yolk sac. The main arteries and vein rudiments appear by h. 45 at perpendicular directions and their primary organization (a cross at right angles) seems to be under another genetic control.

3. SIMULATION OF THE VASCULOGENESIS PROCESS

We have then solved for the DLA growth in the very same configuration, by admitting a large circular area for the yolk sac, placing a schematic embryo composed of two lobes and two arterial rudiments perpendicular to the A-P axis located in the heart region. While the DLA simulation does not represent the actual process of growth, it does represent a *Monte-Carlo* solution of the laplacian growth process. The difference between the DLA process and the DBM version lies only in the size of the small cut-off. A typical pattern obtained across the yolk sac is given in Fig. 2(b). One clearly observes the formation of the two main vessels surrounding the A-lobe and the two main vessels surrounding the P-lobe. On each main vessel, secondary vessels appear perpendicular to the A-P axis. This spontaneous morphogenesis has a deterministic flavour to it, in that in all simulations, despite the random character of the DLA model, the set of principal vessels surrounding the lobes was obtained and the set of secondary vessels perpendicular to the A-P axis was rather reproducible. This does not come as a surprise, as DLA in a finite medium tends to generate branches that occupy the entire domain of the simulation.[17] In this instance, the angle between the convex vessel that surrounds an embryonic half and the vessel that shoots out perpendicular to the A-P axis is rather reproducible.

4. POSSIBLE VARIANTS OF THE BASIC MODEL

Once it is recognized that the pressure in the flow via the shear stress on the wall is the physical parameter governing the organization of the pattern, different improvements of the model are possible, thus incorporating more specific features of the actual vasculogenesis process. For example, the embryo contour schematized in Fig. 2(b) might not be the embryo itself, but a zone of influence where the value of k is modified by a chemotactant or a hormone (such as angiopoïetin). Defining $k(x, y)$ as a parameter dependent on space coordinates under the influence of a chemotactant or physical constraints, allows the modification of the vessel growth and creates a class of models, coupling dendritic growth and chemotaxis or mechanical strain. Figure 2 could be equivalently obtained by setting $k(x, y)$ equal to zero inside the lobes and to a constant outside. This mathematical feature would allow the modeling of the role of a diffusible chemical inhibitor in the reaction of endothelial cells to shear stress.

Another tedious, if not difficult improvement, would be to consider the larger branches as having a finite resistance. Indeed, the DLA process in its DBM version amounts to a progressive replacement of the small capillaries by a perfectly conducting branching structure. However, the branching pattern is not, strictly speaking, perfectly conducting since the viscous resistance is proportional to the inverse of the cross section ($1/R^2$). As a consequence, in order to be more precise, one should consider the invasion of a lattice of capillaries by a tree of smaller, non-zero resistance. However, this instance has already been extensively discussed in the literature,[18] and it is well known that as long as the invasion proceeds as a less resistive medium invading a more resistive medium, the pattern is a branching structure, which will be all the more space-filling as the resistance of the invading pattern is higher.

Also of interest is the instance in which the vascular network is composed of several planar layers (as seems to be the case in the eye) which are connected through a vertical array of capillaries.[19] In this case, growth in one layer will be modeled by a DLA process incorporating a source term across the plane. This situation has already been encountered and deeply investigated in the modeling of Molecular Beam Epitaxy.[20] It is also known to give more space-filling patterns than DLA.

Capillary regression, as observed in most instances of vasculogenesis will also be easily implemented and it will change the apparent fractal dimension.

5. CONCLUSION

By considering the sensitivity of endothelial cells to shear and the law of incompressibility for the blood, one is able to place a limiting case of vasculogenesis in the universality class of the fractal dendritic growth. Though several aspects deserve further investigation (especially capillary regression, anisotropy of the vascular wall, mechanisms of mechano-transduction, etc.), it is clear that dendritic growth will allow to model important features of the branching process, including such important situations as tumor growth, new vascularization after grafts or wound healing etc. Indeed, even if the pruning mechanism occurs on a growing set of small capillaries (angiogenesis) and not on a well-percolating lattice of capillaries, the basic features of the instabilities are conserved.

Implementing additional or more specific features, such as the shape of the embryo and the yolk sac, or of a given organ, or other features such as dilation during growth, finite viscous drag in the large vessels, capillary regression, etc. will allow to describe more exactly a given situation, still with a dendritic growth model as starting point.

It has been argued that the DLA model should be rejected on the grounds that its fractal dimension 1.7 is not always recovered in analysis of vascular networks.[19] However, it is known that the fractal dimension of a tree growing according to a strict DLA rule will not be 1.7 if the simulation is performed in a finite medium,[17] as is always the case in biological instances; moreover, if there is a source term, or a finite concentration, the fractal dimension changes as the surface coverage of the pattern increases.[20] In the end, it is clear that one should not be too demanding on finding a fractal dimension of 1.7, since the wealth of models based on DLA can give, on implementing additional features, different fractal dimensions.

Let us end by saying that, from a clinical point of view, it looks more likely that a mechanism of growth closer to the original DLA/DBM growth mechanism will be at work during normal vasculogenesis than during tumor growth.

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