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MODELISATION OF 3-D MICROVASCULATURE BY INTERLACED DIFFUSION LIMITED AGGREGATION

VINCENT FLEURY
Laboratoire de Physique de la Matière Condensèe, Ecole Polytechnique, 91128 Palaiseau Cedex, France

LAURENT SCHWARTZ
Service de radiothérapie, Pitié-Salpêtrière, 47 Bd de l'Hôpital, Paris 75651, France

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Abstract

We present a model of 3-D vascular formation which is able to explain how the capillary system matures into two 3-D arborescent vasculatures which interdigitate in the distal parts, without forming direct shunts.

1. INTRODUCTION

While there exist considerable data on the morphology of the vascular networks, the mechanism of microvasculature formation of entire organs is not understood. Observations of vasculatures (such as the ocular vasculature) reveal an intricate pattern of arterioles overlapping venules, venules overlapping arterioles, venules overlapping venules, and arterioles overlapping arterioles. However, despite this intricacy, venules and arterioles interdigitate "naturally" in the capillary bed It would require an enormous amount of data to construct this highly random vasculature in a deterministic way. We present in this article, a simple scenario based on known biological facts and results from out-of-equilibrium physics that appears to explain essential features of vasculogenesis. We propose a simple sequential mechanism of capillary formation, vascular growth with capillary regression, and vascular regression that ends up in an intricate pattern, randomly self-organized, though easy to construct. The process reveals a remarkable economy of means. The model gives a heuristic reasoning for finding the vasculogenetic process of a given organ and prompts new tracks of medical research.

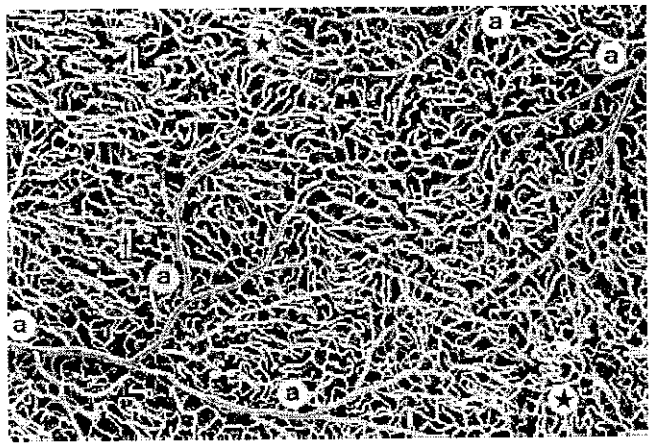


Fig. 1 Araldite cast of a capillary bed in the retina. It can be viewed as a 3-D array of small tubes (*Courtesy of P. Simoens, reprinted from Ref. 4.*)

Fig. 2 Araldite cast of a vascular bed in the choroid, showing the intricacy of the arterial and venal vasculatures. (*Courtesy of P. Simoens, reprinted from Ref. 4.*)

2. BIOLOGICAL AND PHYSICAL BACKGROUND

In an organ (e.g. the eye), microvasculature is composed of several regions, which can be schematically decomposed into two areas. First, a capillary bed where exchange occurs with the surrounding tissue (Fig. 1), and second, a vascular tree composed of larger vessels that drain blood to and from the capillary network (Fig. 2). The vascular network is composed of two sets of vessels — arterioles and venules. Arterioles and venules form a very interlaced structure, composed of two branching patterns that ramify from the main arteries and veins down to the capillary network. It comes as a remarkable observation that venules and arterioles do not, in normal circumstances, flow directly one into the other. The arterioles go all down to the capillaries, and venules emerge from the capillaries. There exist few, if any, shunts from arterioles to venules, except in pathological situations. In many instances, arteries and veins are paired, and follow each other in close vicinity. How such a structure can be constructed remains largely mysterious. It seems impossible that venules or arterioles should have snaked across one another in such a complex way, without connecting to each other, before reaching the capillaries where they meet "miraculously"

It has been proposed recently[1] that the basic mechanism by which vasculature forms during embryogenesis requires in fact transformation of small capillaries into larger vessels by action of the shear stress on the cellular wall. In the model, there only exists, at start, a capillary plexus which can be described formally as a random percolating array of small tubes. Then, smaller vessels transform into large vessels. In this picture, the growth of the vascular tree is not at all an actual growth of a tree, but a progressive selection among small capillaries of what will become the larger vessels. Progressive replacement of small capillaries by larger vessels is able to explain the formation of vessels in the 2-D *area vasculosa* of the chick embryo, whose structure is much simpler than that of organs.[1,2] Much remains to be understood about the molecular basis of the action of shear stress. However, it is well established that shear stress enhances the release of vascular growth factors and the secretion of matrix metalloproteinases which lead to vascular enlargement.[3]

3. THE 3D MODEL

In this letter, we discuss the case of a 3-D organ, taking as an example the ocular *vasculosa* for which detailed Araldite casts of (pig, canine and feline) vasculature are available.[4] The basic scenario that we propose is the following. During the early stages of organogenesis, a 3-D lattice of small capillaries forms. There are no veins or arteries, except for some rudiments that make a *way in* and *a way out* for the blood flow. The formation of this lattice is entirely random and akin to a bond percolation process.[1,5] The "bonds" correspond to small isolated islands of blood that form randomly until percolation occurs. This is consistent with observations in the chick embryo, and also with casts of vessels (Fig. 1).

As the tubes percolate, circulation becomes possible and small capillaries will progressively transform into larger vessels. This is where out-of-equilibrium physics comes into play. We shall make the simplifying assumption that the large vessels are all much larger than the capillaries, and that they are all of the same diameter. In brief, tubes of a small radius r enlarge and become progressively tubes of diameter R, with $R \gg r$. We shall take the shear stress as being the primary agent of the enlargement of capillaries.[1,2,6–8] In the flow, a pressure field spanning the entire structure satisfying the equations of hydrodynamics in the tubular network is established. In a first approximation, and neglecting pulsation, the flow V in a given segment of capillary is proportional to the drop of pressure between the two ends, to the square of the cross-section and to the inverse of the viscosity.[9] For an equal pressure drop, flux is much higher in large vessels than in small vessels, the viscous resistance being considerably smaller. The pressure field in the entire plexus can be solved by solving the Laplace equation across the lattice of bonds. (This corresponds to fluid conservation.) In our model, the enlargement of the vessels occurs in the direction of high shear, *i.e.* in the directions of high pressure gradient. The growth speed of the vasculature v (not the fluid speed V) is then proportional to the gradient of the pressure P. In the end, the growth of the large vessels "across" the capillary bed is modeled by the following equations:

$$\Delta P = 0 \text{ in the capillaires}$$

where Δ is the laplacian operator $\partial_x^2 + \partial_y^2 + \partial_z^2$

$$P = P_0 \text{ in the arteries.}$$

$$P = P_1 \text{ in the veins.}$$

$$v = -k.\ \mathbf{grad}\ P$$

This model is identical to the Diffusion Limited Aggregation (DLA) model,[10] in its dielectric breakdown version.[11] The analogy with dielectric breakdown sparks is as follows: the growth of the vascular tree is formally identical to an electric spark propagating on a network of resistances, the capillaries correspond to segments of high resistivity, and the larger vessels, in the analogy, correspond to the spark itself, *i.e.* a region of very low resistivity. It has been known for some time in statistical physics,[12,13] that this growth process belongs to a class of out-of-equilibrium self-organized processes leading to hierarchical branched structures.

Now, while this mechanism is able to explain the formation of an arterial bed in a 2-D primary plexus,[1] it is still insufficient to explain the pattern of, say, the ocular *vasculosa*, because in this instance, there exist in the same zone large venules *and* arterioles that intertwin, and a capillary bed further down. Venules and arterioles interdigitate and irrigate the *same* region in the capillary bed. This fact is impossible to explain with the basic "dielectric spark" model described above, when *two* vascular trees and *two* "sparks" must be considered. This is why we need an additional essential fact, which is indeed observed in real vasculogenesis — capillary regression.[6] As the vessels penetrate into the capillary bed, the capillaries that are connected to them regress and disappear.[1,6] This does not come as a surprise, since there is almost no flow in them. Eventually, vessels connect to the capillary bed by the tips and not by the sides of the tubes.

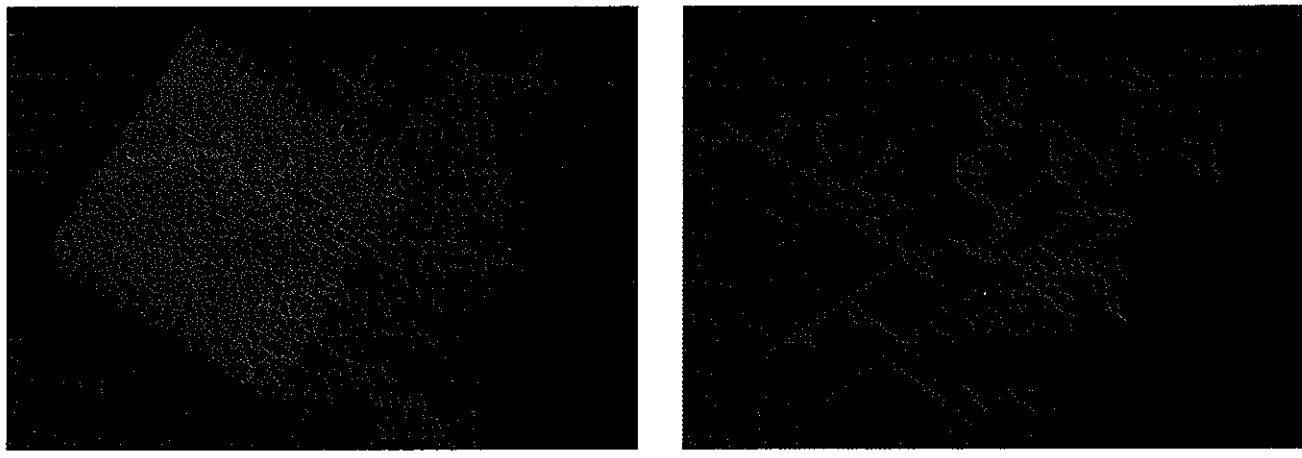
Now we show that this feature, regression of capillaries wherever the shear is too low, is sufficient to construct a model of 3-D vasculogenesis of an entire organ as follows. Starting from an arterial rudiment, a first tree forms in a 3-D plexus, which then grows following the shear stress in a DLA fashion. Capillary regression disconnects the tree from the capillaries, except at the tips. (It is likely that this occurs progressively during growth.) Let us note that the remaining plexus resembles the primary plexus, except for links that have been cut off. From the topological point of view, there is little difference with the primary plexus, because capillaries are markedly more numerous than vessels (compare Figs. 1 and 2). Mathematically speaking, the set of bonds making the tree has zero measure inside the set of all the bonds, because it is a fractal. Now, this capillary plexus serves for the growth of a second tree — the venal tree. Starting at some point from a venule rudiment, and progressing in the same way, a second tree will grow across the same 3-D plexus, ignoring the presence of the (disconnected) arterial tree, and ending in the capillaries which do not transform into large vessels. Eventually, the capillaries disconnect from the main veins, again except at the tips. The final aspect of the vasculature decomposes into two regions: (1) The capillaries that did not transform nor disappear, where exchange occurs, (2) The region of vascular trees, from which capillaries have disappeared. This region shows a complex interlace of

arterioles and venules, with no apparent logic, since arterioles and venules have ignored each other during growth. One will observe venules overlapping venules, venules overlapping arterioles, etc. However, if considered independently, both trees will branch in an analogous fashion, and grow towards the capillary bed where they interdigitate, since both arterioles and venules are formed from a subset of tubes that, in the first place, were percolating. Despite the apparent randomness, the venules will statistically grow towards arterioles causing a statistical "pairing" because the source term of P, which drives the growth of the venules, is at the tips of the arterioles, where blood flows in. This "veins towards arteries" tropism is scale invariant.

We have implemented numerically this process. The result [Figs. 3(a)–(b)] shows all the features evoked above. In the end, the process reveals a remarkable economy of means in that:

1. It uses only local biomechanical information, in the form of the local shear.
2. Since it uses the shear flow as long range information, it constructs the vasculature in the regions of actual flow.
3. It uses the same capillary bed for arteries and veins.
4. It preserves the vital demands during construction (compatible with ontogeny), and it can and also be evolved easily (compatibility with phylogeny), since progressive improvement of the feedback loop "shear-sensing ⇒ capillary transformation" will progressively form improved vasculatures.

The mechanism proposed here has long been shrouded by the fact that capillary regression destroys the true "medium" in which vascular trees (which is the random percolating lattice of capillaries) are formed. When one observes a given



(a) (b)

Fig. 3 (a) Numerical simulation of microvascular growth in a large 3-D "organ". Initially, there exist only small capillary tubes (green lines, only one-fourth shown). An arteriole rudiment is put at the left of the capillary bed, and it is allowed to grow by the DLA process,[1,10–12] which is a Monte-Carlo simulation of growth in the direction of high shear stress. An arterial vasculature progressively grows (red bonds). We have interrupted the process when 500 capillary segments had been turned to arterioles. Then, capillary regression disconnects the arterial vasculature, except at tips. As usual in DLA processes, the tips are the last formed bonds. It is then very easy to select the tips of the arterial vasculature, as say, the 50 last formed arteriole segments. Then, a venal tree grows, starting from a venule rudiment (put close to the arterial rudiment), well into the capillary bed, in a random digitating fashion, which snakes spontaneously towards the tips of the arterial vasculature. In the end, one gets two well branched 3-D vasculatures, that have both grown naturally into the capillary bed by following the directions of high shear stress, and which interdigitate naturally. The image was processed with the MolMol software of IMB Zürich, it shows the entire set of arterioles and venules that the process has generated. For the sake of clarity, the simulation (in language C) was limited to $40 \times 40 \times 7$ capillary segments, it takes about 10 secs on a Sun Sparc station to run the program, and (b) The set of vessels after *vessel regression* (not capillary regression). We suppose that, in the long term, all the dandling "dead-ends", *i.e.* veins and arteries that do not end in one of the 50 tips disappear. We have removed those. There remains a plausible vasculature, entering at one end, and irrigating mainly the distal end of the capillary bed. Note that venules have (statistically) grown towards the arterioles. There exist few loops.

vasculature, and tries to derive a reasoning for their construction, one must first imagine an entire plexus *in lieu* of the observed vasculature, and then place the limiting conditions that will allow the observed vasculature to emerge from the selection mechanism explained above. Additional features, such as a flow resistance depending on cross-section, presence of several incoming rudiments, or a finite time delay before capillary regression, should also be considered. However, the basic model presented here allows to generate a realistic vasculature, as in Fig. 3, comprising an enormous quantity of capillary segments. To our knowledge, it is the first model that allows construction of a vasculature of a 3-D "organ" from nil. Existing models,[14] which tried to implement similar features, failed to produce branching vasculatures because either they allowed for reversibility of the vascular change, or they did not stop the enlargement process in the zone of hypoxia.

It is likely that the mechanism described here guides angiogenesis after cardiac infarct to compensate for hypoxia. Sprouting of new capillaries towards the existing capillaries opens new stream pathways which allow further remodeling of the vascular bed by the action of shear stress.

On the other hand, angiogenesis induced by cancer cells is clearly abnormal; endothelial cells migrate towards the tumor, proliferate to form nascent closed capillaries with the ultimate appearance of a lumen.[15] We hypothesize that these capillaries do not respond normally to shear stress, resulting in the observed leaky capillaries and arterio-venous shunts.

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