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Intimate contact between red blood cells and vessel walls is sufficient to stabilize capillary networks during development

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Blood flow | Microcirculation | Vascular morphogenesis

Hierarchical branching networks are ubiquitous around us in nature, including river networks, lightnings, trees and their root systems. Most of them form transportation networks. Some have a convergent architecture transporting for example water from the smallest streams to the largest rivers. Others have a divergent architecture transporting for example electrons from clouds to ground throughout an increasing number of branches. Trees combine a convergent root system under ground with a divergent one above ground, where sap flows from trunk to leaves during evapotranspiration. In general, such hierarchical branching networks are consistent with the constraints that the time for supplying resources as well as the length of distribution pathways should be minimized. However, because of the large distances separating their terminal branches, they are not optimal for the delivery of goods to distributed locations that are filling a space, such as buildings in a city center. That's why human-built logistic networks, including roads and water-distribution and waste-collection systems, mostly combine branching networks (e.g. freeways, highways and large avenues) with interconnected, loopy ones (smaller streets enclosing city blocks).

A striking natural example of such a combination of branching and interconnected networks is the blood vascular system of vertebrates, including fishes, birds, and mammals (Fig. 1). This system is able to ensure adequate blood supply, feeding every cell in peripheral tissues with oxygen and nutrients under a wide range of physiologic conditions and varying metabolic needs. For that purpose, it is comprised of three distinct families of vessels, functionally organized in series (Fig. 1A,B): 1/ the arteries, which collectively form a divergent tree that carries blood away from the heart; 2/ the capillaries, the smallest vessels in the body with sizes typically matching or smaller than that of individual red blood cells (RBCs), which together form an interconnected (or loopy) network connecting the arteries to the veins; and, 3/ the veins, which carry blood back to the heart through a convergent arborescence (1–3). Blood flow has been known to be a primary driver of vascular network growth since the pioneering work of Thoma in 1893 (4). Schematically, the cells lining the walls of high-flow vessels (endothelial cells) are submitted to high friction forces, which trigger them to reorganize (by migrating and changing shapes/orientations), leading to vessel enlargement, and vice-versa (5, 6). However, this simple mechanism essentially leads to the extension of trees in the vascular network, with concurrent regression of vascular loops, giving rise to direct connections, or shunts, between arteries and veins (5, 7–9). In PNAS, by combining mathematical models of vascular adaptation and experiments in the zebrafish embryo (Fig. 1D), Qi et al. (10) thus address the long-lasting and unresolved question in vascular

physiology: what are the underlying mechanisms for the stability of loopy vascular structures, characteristic of capillary beds, in microvascular networks? In such capillaries, Qi et al. (10) underline that the friction forces strongly fluctuate in time and space, being maximal when RBCs, which typically flow in single files in these small vessels, are passing by. Taking inspiration from network physics, where fluctuations have been put forward to explain the coexistence of branching and loopy structures (11), their central hypothesis is that diameter changes in capillaries are driven by these peak friction forces.

Previous experimental work elegantly demonstrated the involvement of blood flow in the proper formation of the branching microvessels. For example, in chick embryos, suppressing blood circulation after heart removal prevented the growth of arteries and veins, despite the capillary network continuing to develop for several days (1). Manipulation of blood flow, for example by ligation of an artery, dramatically affected the final vascular pattern (Fig. 1C) (1, 12). In mouse embryos, sequestering RBCs out of the blood vessels also prevented the growth of branching vessels, while restoring the viscosity by adding biopolymers, which mainly increases the average friction, restored normal vascular development (6). However, theoretical models considering the rate of enlargement of blood vessels being proportional to average friction fundamentally lead to unstable solutions for two vessels organized in parallel (7). In other words, for this minimal representation of a loopy network, the underlying mathematical structure representing this simple problem is unstable, inevitably leading to one of the vessel vanishing while the other one enlarges (7, 10). In larger networks, this progressively drives the regression of all capillaries, with the ultimate collapse of all remaining branches into a single pathway that connects the main arterial inlet with the main venous outlet (5, 7, 8). This fundamental gap in our understanding of vascular development has been called "the shunt problem" (9).

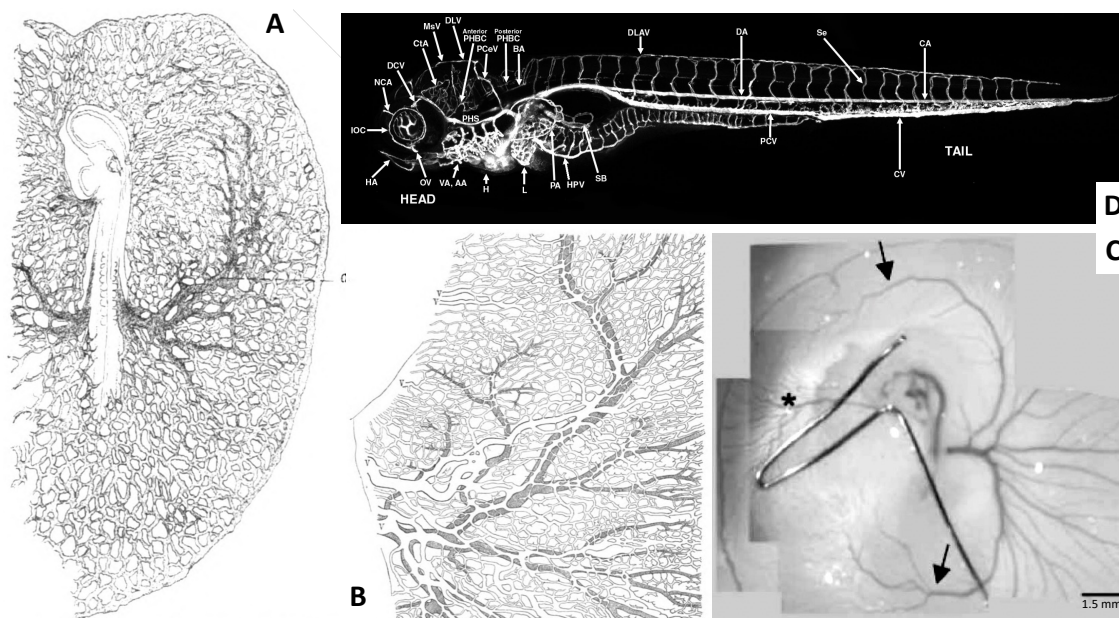
Qi et al. (10) first mathematically rewrite the simplest minimal problem for the diameter evolution of two parallel vessels, with peak friction now being the driver. For that purpose, they make the following assumptions: 1/ a simple geometrical model can describe how spherical particles, representing RBCs, are squeezed in vessels with diameter smaller than their undeformed diameter; 2/ a thin liquid film with approximately constant thickness is trapped between their squeezed portion and the vessel wall, in which the local friction is much higher than the activation value triggering the reorganization of endothelial cells; 3/ the fraction of activated endothelial cells in each vessel is thus proportional to the total

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Fig. 1. Microvascular architectures combine branching and interconnected (or loopy) networks. A-C: Chick embryo yolk-sac; A,B: Drawings of vessels by (4). Arteries are in black, veins and capillaries in white (B: enlarged view); arteries and veins are often found paralleling to each other, but are generally not directly connected, except through the capillary bed; C: Ligature of the left hand side artery by a metallic pin yields to its venularization (*) and to increased arterial growth on the opposite side (arrows). Reproduced from (12) with permission. D: Zebrafish embryo (4.5 dpf) from (13); here, the caudal (tail) region of the DA and PCV are labeled as caudal artery and vein (CA and CV, respectively). For a full list of abbreviations, see <https://zfish.nichd.nih.gov/>.

length of this liquid film, i.e. summing over all squeezed RBCs in this vessel, divided by the vessel length, and $4/\lambda$ the rate of vessel enlargement is proportional to the difference between this activated fraction and an activation threshold. They show that the simplest associated mathematical problem of vascular adaptation driven by peak friction leads to a stable equilibrium point, in which both parallel vessel converge to an equal radius.

With this result, Qi et al. (10) move on to a more complex network structure representing the main vessels in the tail of the zebrafish embryo four days after fertilization. In doing so, they focus their attention on the subsequent evolution of a single pair of artery and vein, the dorsal aorta (DA) and the posterior cardinal vein (PCV) and their interconnecting ladder-like capillary-sized vessels, the intersegmental vessels (Se-vessels) (Fig. 1D). Keeping the above assumptions, they also introduce the unequal repartition of RBCs downstream microvascular bifurcations, with more RBCs generally biased toward the highest flow vessel (14), as an additional ingredient. Whatever the model used to represent this phenomenon, their peak friction vascular adaptation model yields increasing Se-vessel diameters and increasing RBC concentrations from head to tail when starting from a uniform or random affectation of the diameters of Se-vessels, consistent with their experimental observations at four days. These systematic variations compensate for the decreased pressure gradient that establishes across each successive Se-vessel due to the increasing distance from the network inlet, and ensure uniform perfusion of RBCs from head to tail, once again consistent with the experiments. In contrast, adaptation driven by average friction would lead to the opposite trends, leading to a large shunt depleting the tail region from blood and RBCs. Thus, peak friction forces provide a simple mechanical cue stabilizing the loopy Se-vessels in the tail region, and, in the same time, ensuring uniform RBC perfusion throughout the tail.

However, this mechanism requires a sufficient number of RBCs in each vessel for the endothelial cell activation fraction to stay higher than the activation threshold. If not, the vessel will regress to a size too small for RBCs to squeeze in, potentially disrupting perfusion uniformity. Qi et al. (10) thus make the assumption that, between 7 and 15 days after fertilization, when the amount of RBCs in blood drops possibly due to RBC maturation, uniform perfusion must be lost. They first experimentally measure RBC fluxes from head to tail during this time frame, and confirm almost complete loss of RBC perfusion in the four Se-vessels closest to the head at day 10. Then, keeping the same starting network as previously, they reproduce their computations of peak friction force adaptation with smaller and smaller values of the inlet RBC concentrations, mimicking the observed evolution. They show a complete loss of RBC perfusion on these same vessels after ten days, combined to a lower value of their diameters due to the associated drop in the activation fraction. Noteworthy, just reducing the inlet RBC concentration while keeping fixed values of the diameters (no adaptation) does not lead to vanishing RBC perfusion in the head-most Se-vessels. Once again, adaptation driven by average friction cannot either explain this observation.

Finally, as increasing concentrations of RBCs from head to tail emerged as a central ingredient of loop stabilization as well as uniform perfusion, Qi et al. (10) further examined whether a simple experimental manipulation could affect the distribution of RBCs. In doing so, they realize that, due to RBCs being biased toward the highest flow vessel at bifurcations, a majority of them remains in the DA after each successive intersection with Se-vessels. A large proportion of them therefore continues directly downstream to be drained by the PCV. They conclude that amputating the tail to ablate the direct connection between the DA and PCV at the tail should have a strong effect on both the RBC distribution in Se-vessels and vascular adaptation. Indeed, performing this experiment, they

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qualitatively show a strong increase of the number of RBCs in the tail-most Se-vessels. Consistently, the adaptation model predicts a large (~ 5 -fold) increase of the RBC concentration in these vessels, which consistently leads to increased diameters. In combination, this leads to a strong increase in RBC perfusion towards the tail. Uniform perfusion is retrieved by the model if the conductance of the DA/PCV connection is progressively restored, with a diameter matching that of the real zebrafish embryo.

Overall, by combining experiments in the zebrafish embryo, including manipulation of blood flow by ablating the DA/PCV connection, highly idealized mathematical models of vascular adaptation induced by peak shear stress and more realistic physically-based models integrating some aspects of the complex behavior of blood flow in microvessels, Qi et al. (10) provide strong evidence that vascular adaptation driven by peak friction forces could be a mechanical driver for the stabilization of capillary loops, yielding uniform perfusion. Other mechanisms have been previously proposed to counteract the formation of vascular shunts during development, including capillary disconnection from the larger arteries and veins (12, 15, 16), upstream information transfer along vessel walls (8, 9) and metabolic signaling, by which vessel diameter increase if oxygen is locally insufficient to support the metabolic demand (8, 9). However, the two first ones mostly avoid proximal shunting (shunting between large arteries and veins) while the last one is ineffective at early stages of development when the embryo is sufficiently small to get its oxygen by diffusion from the surrounding atmosphere, as underlined in (10).

Of course, this study also opens a wealth of additional questions. In particular, is the peak friction model also relevant for larger vessels or is average friction driving vessel remodelling there? If yes, what is the cut-off size between these two regimes? The ideas exposed in (10) suggest it would correspond to the transition between single and multiple file RBC motion in small vessels (14). This transition also roughly corresponds to a minimum in the viscous dissipation for blood flow in small vessels. Does this contribute to setting up the diameter of vessels at the transition between branching vessels and loopy ones in the vascular hierarchy and/or to minimizing the cost of blood distribution? On

another side, the results obtained by (10) pinpoint the importance of the connection between the DA and the PCV at the end of the tail. As these connecting vessels establish as soon as the heart begins to beat around 24h postfertilization in the zebrafish embryo (13), and at that time are the only ones in the trunk and tail regions, this raises the question of the mechanisms driving this initial, large-scale circulatory loop. Previous work in the chicken yolk-sac suggested that growth of the yolk-sac induces tissular pressure gradients contributing to the formation of the initial arterio-venous pattern (17). In the brain, this initial pattern has been suggested to be involved in the formation of large penetrating arterioles and ascending venules in the cortex (16). Importantly, up to now, the initial organization of this large-scale pattern must be postulated as a starting point in modeling studies of vascular development, in the yolk-sac as well as in the brain (12, 18). Combined with emerging experimental observations of the postnatal development of the capillary bed in complex tri-dimensional organs, including the brain (19), this opens the perspective to understand whether the present results are generalizable. Finally, how the above mechanical cues interplay with the genetic and molecular machinery that drives the reorganization of endothelial cells and sets up vessel identities (1, 6) still needs to be uncovered.

Answering these questions will be key to understand how dysregulations of this interplay leads to impaired microvascular architecture and function in a large range of diseases, including cancer, diabetes, neurodegenerative diseases, as well as normal aging, possibly leading to novel diagnosis and/or therapeutic strategies. Moreover, it will help overcome our current inability to engineer organoids with functional vasculatures, a fundamental bottleneck that strongly limits their size and their maturation potential beyond the developmental phases (20). This will open future opportunities, especially in the field of organ replacement.

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