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# Systems Biology of the Microvasculature

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## Abstract

The vascular network carries blood throughout the body, delivering oxygen to tissues and providing a pathway for communication between distant organs. The network is hierarchical and structured, but also dynamic, especially at the smaller scales. Remodeling of the microvasculature occurs in response to local changes in oxygen, gene expression, cell-cell communication, and chemical and mechanical stimuli from the microenvironment. These local changes occur as a result of physiological processes such as growth and exercise, as well as acute and chronic diseases including stroke, cancer, and diabetes, and pharmacological intervention. While the vasculature is an important therapeutic target in many diseases, drugs designed to inhibit vascular growth have achieved only limited success, and no drug has yet been approved to promote therapeutic vascular remodeling. This highlights the challenges involved in identifying appropriate therapeutic targets in a system as complex as the vasculature. Systems biology approaches provide a means to bridge current understanding of the vascular system, from detailed signaling dynamics measured *in vitro* and pre-clinical animal models of vascular disease, to a more complete picture of vascular regulation *in vivo*. This will translate to an improved ability to identify multi-component biomarkers for diagnosis, prognosis, and monitoring of therapy that are easy to measure *in vivo*, as well as better drug targets for specific disease states. In this review, we summarize systems biology approaches that have advanced our understanding of vascular function and dysfunction *in vivo*, with a focus on computational modeling.

## 1. INTRODUCTION

### 1.1 Motivation for a systems approach to the vasculature

Systems biology is an integrative approach that synthesizes our current understanding of molecular, physiological and pathological mechanisms to reconcile experimental data from multiple perturbations with the predictions of detailed computational models. By integrating detailed experimental data (e.g. from hi-throughput experiments) with mechanistic information (e.g. from multi-scale computational models and bioinformatics), we can formulate a more complete understanding of a system across multiple scales and at higher spatial and temporal resolution than would otherwise be possible. In addition, modeling the interconnectedness of the system from gene to protein to pathway, and from cell to tissue to organism, allows systems biology simulations to predict the system-wide response to perturbation, for example the change in blood supply to a tumor following delivery of drugs.

Systems biology is well-suited to studying vascular function and dysfunction because the vasculature and its regulation are highly complex. The insides of all blood vessels – from the smallest to the largest; arteries, veins, capillaries; newly sprouting or mature – are lined with endothelial cells (ECs). This cell type must therefore be sufficiently flexible to survive and thrive in diverse environments, and to perform different specialized functions in many tissues[1]. In particular, moving from *in vitro* systems in which perturbations to endothelial cues can be controlled to *in vivo* vascularized tissues necessitates a quantitative understanding of these complex systems. Whether following exercise[2] or in a growing tumor[3], there can be changes to the expression of many or all of the ligands and receptors regulating endothelial cell behavior, and not all in the same direction. The outcome of all of these changes would be impossible to calculate without a detailed quantitative model of the system.

Because of the number of potential levers and drivers of vascular changes, there are many possible quantitative metrics to measure, including potentially informative quantities that are difficult to measure *in vivo*. By incorporating detailed *in vitro* measurements, computational models can be validated and used to identify which *in vivo* measurements would be most informative – as diagnostics, prognostics, or as indicators of therapy effectiveness either before or after treatment.

### 1.2 Vascular development and remodeling

The vasculature supplies oxygen to tissues. Maintenance of homeostasis requires the vascular system to adapt in response to local stimuli (e.g. oxygen tension) sensed by endothelial and other cells. The smallest vessels, directly involved in delivery and transport of oxygen to tissues, develop new branches, expand in diameter, or are pruned as a result of these dynamic molecular, cellular, and tissue microenvironmental cues (Fig.1). Vascular network development, maintenance, and remodeling can occur through multiple distinct morphogenic processes. Each requires complex molecular and multicellular regulation, though the regulatory details are not completely understood for any of these forms of vascular remodeling.

Early in development, blood islands coalesce and lacunae form, resulting in a network of interconnected endothelial cords[4]. This process, by which whole networks can be formed simultaneously, is known as **vasculogenesis**. The tendency of ECs to coalesce and form cords in this way has been leveraged for *in vitro* assays[5], and studied using computational models of early vascular network formation by the Glazier group[4, 6].

Following vasculogenesis, the blood vessel networks in developing organs must be refined and expanded as tissues grow and differentiate. The process of **angiogenesis** increases vascular density by sprouting new vascular branches or splitting existing vessels in two. Sprouting angiogenesis takes two forms: first, expansion of vascular networks into currently avascular tissue – for example, the perinatal expansion of the retinal vasculature[7, 8], or the investment of new vessels into small tumors; second, the dynamic sprouting and pruning/regression of vessels within an existing network[9], for example due to exercise or within a growing organ. In both forms of sprouting angiogenesis, endothelial cells become activated by stimuli secreted from distant cells and undergo phenotypic differentiation to migratory,

vessel-sprout-leading ‘tip’ cells. These cells degrade local extracellular matrix and lead proliferative stalk ECs to form sprouts that may ultimately anastamose and become part of the blood flow circuit. Intussusceptive angiogenesis is different to sprouting: existing endothelial tubes form internal pillars that lead to splitting of one vessel into two. This form of vascular expansion can result from changes to shear stress[10, 11].

Vasculogenesis and angiogenesis are both typically processes of microvessel development. To obtain hierarchical vascular networks, growth (diameter expansion) is required. **Arteriogenesis** is the process of expansion of existing arterioles into larger vessels[12], permitting the vessel to carry more blood flow. **Capillary arterialization**[13], also known as arteriolargenesis[14], is the process by which capillaries can, under specific circumstances, expand beyond typical capillary dimensions and acquire the characteristics of arterioles. Diameter expansion is typically accompanied by the acquisition of arterial/venous phenotype, including the investment of perivascular smooth muscle cells (SMCs)[14].

Incorporating current understanding of the different vascular remodeling processes (Fig.1) into systems biology approaches is important for identifying proper strategies to promote or prevent vascularization in disease applications with distinct vascular network morphologies. The main drivers of these processes vary, including different local mechanical and chemical cues sensed by ECs. This suggests that multiple types of therapeutic targets may be combined to selectively activate or inhibit one or more of these remodeling processes. In this review, we focus primarily on non-developmental vascular remodeling, specifically discussing sprouting and intussusceptive angiogenesis. To date, arteriogenesis and capillary arterialization have not been the subjects of significant systems biology efforts; these provide opportunities for future work. **Section 2** will provide more detail on the types of models used to study different vascular remodeling processes, and the components included in these models. **Section 3** will discuss the use of systems biology to identify effective therapeutic approaches to stimulating or inhibiting the vasculature. **Section 4** will highlight challenges and bottlenecks that must be addressed to translate advances in microvascular systems biology into improved clinical outcomes.

## 2. MICROVASCULAR SYSTEMS PHYSIOLOGY AND PATHOLOGY

Vascular development and remodeling *in vivo* comprises several multicellular, multi-scale morphogenic processes. A systems approach is required to understand these processes and the effect of physiological and pathological changes to the system. In this section, we will describe the multiple scales of integrated regulation involved in vascular remodeling (Fig. 2). While the goal is to improve clinical outcomes in disease, our ability to measure systems changes *in vivo* is often limited. As such, computational studies of molecular and cellular regulation rely heavily on *in vitro* experimental studies for validation. These results must then be interpreted or translated to an *in vivo* context to be used for biomarker development and prediction of therapeutic responses. Appropriate computational models can provide this bridge between *in vitro* and *in vivo* measurements. For a detailed review of the mathematics underlying many of the modeling techniques presented here, see[15].

### 2.1 Blood flow and oxygen distribution: a system of delivery and consumption

The vasculature comprises a hierarchical network of interconnected endothelium-lined tubes. The flow of blood distributes oxygen to tissues, with local blood flow matching metabolic demand[16]. Mismatches in blood flow and tissue oxygen consumption can arise from normal processes such as growth and exercise, as well as pathological conditions including stroke, diabetes, respiratory disease, and myocardial infarction[17]. Mathematical models of blood flow fall into two categories: (a) three-dimensional models of blood flow, particularly potentially turbulent flow at sites of atherosclerosis in large vessels[18, 19]; (b) network models of blood flow in systems of smaller vessels, in which laminar flow permits the use of Poiseuille-based algebraic models. One example of the first category of models is work by the Diamond group, which integrates hemodynamics with signaling cascades in platelets[20] and stochastic models of coagulation initiation[21] to study the effect of hemodynamics on blood components, including red blood cells and platelets. These models allow for prediction of clot formation and drug sensitivity under varying platelet signaling and flow conditions[22]. The network models in the second category can incorporate experimental measurements of heterogeneous and dynamic microvessel diameters, pressure, flow rates, shear stress, and oxygen exchange[23]. Shear stress and local oxygen availability in particular are key stimuli for angiogenesis and remodeling of the vessel wall, for which predictive models have been developed by the Secomb and Pries groups[24, 25]. Combined experimental-computational systems studies such as these can produce interesting predictions with implications for *in vivo* physiology and pathology, such as that the vascular wall must be capable of sensing oxygen levels in order match experimental observations after changes in blood flow and oxygen distribution[26].

The biomechanics of blood flow are important for intussusceptive angiogenesis. While this form of angiogenesis has not been studied as extensively as sprouting angiogenesis[11], intussusception is thought to be the primary form of vascular remodeling in animal models with vascular endothelial growth factor (VEGF) overexpression[27], chronic shear stress[28], or colitis[29]. Computational models have demonstrated that hemodynamics and shear stress[30–32], along with oxygen consumption[33] contribute to vessel splitting and pillar formation, which are requirements for intussusceptive angiogenesis. Szczerba *et al.* generated the first model incorporating the combined effects of hemodynamics, chemical agents, and vessel wall stiffness on intussusceptive angiogenesis[10]. In this model framework, increasing vessel wall stiffness during development (a result of pericyte investment and/or basement membrane deposition) was required to produce realistic predictions of vessel splitting[10]. Interestingly, another computational model (of skeletal muscle) predicted that intussusceptive angiogenesis can more effectively maintain oxygen levels than sprouting angiogenesis when oxygen consumption is high[33]. Tumor vessels have also been shown to undergo intussusceptive angiogenesis after treatment with angiogenesis inhibitors, but a model of these processes has not yet been developed[34].

Other computational models focus on oxygen distribution in tissues, which is regulated by blood flow, oxygen consumption, and by chemical signal molecules such as nitric oxide. By integrating a blood flow model with an oxygen diffusion/consumption model, the Popel group created a multi-scale model of oxygen transport in skeletal muscle, demonstrating the

influence of muscle fiber type on oxygen distribution[35]. The simulations predicted that the distribution of muscle fiber sizes has a larger impact on $O_2$ distribution than $O_2$ consumption, myoglobin concentration or oxygen diffusivity[35]. Regulation of oxygen by nitric oxide, which stimulates vascodilation and is required for normal endothelial function, has been simulated[36], but this has not been modeled in the context of angiogenesis. The effect of tissue oxygenation on wound healing has also been modeled[37]. More detail on the modeling of oxygen distribution in the microvascular circulation can be found in[38] and[39].

In a later section, we will discuss the importance of blood as a communication route for key proteins and drugs regulating vascular remodeling, as well as the centrality of blood measurements as clinically-relevant, reproducible biomarkers.

### 2.2 Molecular regulators of endothelial cell behavior and vascular remodeling

In the adult, mismatch of oxygen supply and demand can result in changes to the vascular network (Fig. 3), typically through the transcription factor hypoxia inducible factor (HIF) and the vascular endothelial growth factor (VEGF) family of extracellular ligands[40], though other transcription factors and ligands are known to regulate vascular remodeling[41, 42]. Cancer, ischemia, diabetes, and other diseases alter gene regulation, protein expression, and signaling pathway function in angiogenesis, but these changes and their effects on vascular homeostasis are not yet completely understood[3, 43]. As examples, expression of cell surface receptors becomes heterogeneous in many solid tumors due to non-uniform oxygen pressure (resulting from structural abnormalities in tumor vessels)[44]; and changes in shear stress (e.g. due to elevated blood pressure) can alter endothelial gene expression[11].

Normal oxygen levels (normoxia) enable the hydroxylation of the transcription factor HIF1α by prolyl hydroxylases, resulting in HIF degradation[40]. Low oxygen (hypoxia) stabilizes HIF1α, which moves to the nucleus and activates transcription after binding HIF1β/ARNT[45, 46]. There are hundreds of downstream targets of HIF, notably members of the VEGF ligand and VEGF receptor families[46]. Multiple microenvironment-dependent HIF-1α signaling profiles (switch-like or gradual) have been demonstrated using computational models of HIF-1α regulation[40, 47]. Such divergent system behaviors are difficult to couch in a single framework without the use of computational methods. Models of HIF-1 have also been used to: determine the mechanisms through which HIF-1α senses oxygen[40, 48]; study the regulation of HIF-1α[49, 50]; and examine differences in HIF-1α regulation in cancer and ischemia[51] with the goal of identifing promising therapeutic targets for different disease states.

While a wide variety of growth factors, adhesion molecules, and cell-cell communication proteins are involved in angiogenesis, including integrins, cadherins, Delta-Notch and semaphorins, we focus here on VEGF and fibroblast growth factor (FGF); as diffusible proteins that can be measured in the blood, they hold promise for validating predictive models of their transport and impact on vascular behavior.

The VEGF family of growth factors are critical regulators of both physiological and pathological angiogenesis, promoting endothelial cell survival, proliferation, and migration. There are five ligand genes, each with splice isoforms. These ligand genes and splice

isoforms have varying affinity for the three VEGF receptors (which can hetero- or homo-dimerize upon ligand binding), two main VEGF coreceptors (the neuropilins), and the extracellular matrix (ECM)[52]. The VEGF receptors (VEGFRs) also exist as soluble and membrane-bound isoforms[53]. Recent work has demonstrated that post-translational modification (glycosylation, acetylation, methylation) can also modulate the activity of VEGFR2[54, 55]. The multiplicative complexity of these ligands and receptors make understanding the spatial and temporal dynamics of the system and predicting reponse to VEGF-based therapies extremely difficult, as is highlighted by the lack of of success to date in VEGF-based pro-angiogenesis clinical trials[56].

VEGF family members are secreted by parenchymal cells experiencing hypoxia, including: skeletal myocytes in exercise; neural and glial cells in retinal development; bone marrow-derived dendridic cells in wound repair; and hypoxic tumor cells[57]. VEGF isoforms diffuse through the extracellular matrix to bind VEGF receptors on endothelial cells. ECM-binding isoforms also become sequestered in tissues, where they can still activate VEGF receptors[52, 58]. The simulation of VEGF-VEGFR interactions and VEGFR-VEGFR coupling has been developed using biophysically-detailed ordinary differential equation models that are first vaildated against *in vitro* experimental data and then applied to *in vivo* scenarios. This allows for much more detailed understanding than would be possible using only *in vivo* data, which typically consists of plasma protein concentrations, plus some genetic and gene expression data. The scenarios examined to date include competition between ligands for binding to multiple receptors[59]; coupling and enhancement of VEGF binding by Neuropilin co-receptors[60–62]; dimerization of VEGF receptors[63]; downstream signaling of the Akt and ERK pathways[64, 65]; matrix-immobilized growth factors and VEGFR trafficking and phosphorylation[66, 67]. In addition to these detailed models of VEGF dynamics, models have been developed to directly predict VEGF production in skeletal muscle based on oxygen levels, both after exercise and in peripheral artery disease[33, 68–70]. These models allow comparison of disparate therapeutic strategies including exercise and VEGF delivery. Here, exercise was predicted to improve VEGFR ligation and VEGF gradients in ischemic tissue better than therapeutic delivery of VEGF; we will discuss the models of VEGF and exercise as therapies more in **Section 3**. More detail on the systems biology of VEGF can be found in[52, 71].

The fibroblast growth factor (FGF) family has also been implicated in control of angiogenesis. FGFR signaling is complicated by the existence of multiple FGF ligands and the requirement for cell surface heparin sulfate proteoglycans (HSPGs) to stabilize FGF ligand-receptor complexes. A variety of computational models have been developed to study FGF ligand-receptor binding and regulation by HSPGs *in vitro*[72–75], showing that HSPGs able to form active FGF2-HSPG-FGFR signaling complexes are required for effective downstream signaling[76]. FGF binding to EC receptors and to the vascular basement membrane under physiological flow conditions has also been simulated, both *in vivo*[77, 78] and in the context of a bioreactor[79, 80]. These models have quantified variation in FGF-receptor binding as a function of flow conditions, FGF delivery method (bolus or continuous flow), HSPG and FGF receptor density, and binding affinities[77, 79, 80]. In particular, Filion et. al. showed that after intracoronary administration, myocardial deposition and retention of

FGF2 is limited by the time required for FGF to bind cell surface receptors, and not by diffusion[78]. Additionally, they showed that the production and internalization rates of FGF receptors are important in regulating FGF distribution. These results have implications for the therapeutic delivery of FGF, and can be used to predict clinically relevant measurements that are difficult to obtain *in vivo*.

While the majority of systems biology techniques leverage computational methods, the use of systems biology principles in experimental data collection is increasing, and greatly enhances our understanding of the regulation of complex systems. In one example of such work, the lab of George Davis performs high-throughput experimental assays on endothelial cells cultured in the absence of serum[81–83]. This allows for the comparison of many experimental conditions in a well-controlled system, without the variability and background signaling generated by serum typical of most *in vitro* experiments. The angiogenesis and vasculogenesis assays performed by this group[5] have clearly identified the minimal factors required for endothelial tube formation, identifying the key nodes in these complex regulatory networks. Such assays can be compared directly to computational models of *in vitro* sprouting angiogenesis and vasculogenesis, and then scaled to an *in vivo* context.

### 2.3 Vascular remodeling is a multicellular process

In translating extensive experimental results from ECs studied *in vitro* to understanding how endothelial cells behave *in vivo*, we must recognize the different environment that cells have in tissues – a multicellular environment where heterotypic neighbor interactions are key. Vascular remodeling requires the coordinated action of many endothelial cells and their neighbors. In sprouting angiogenesis, VEGF stimulation upregulates tip cell expression of Delta-like ligand 4 (Dll4)[84]. This results in activation of Notch in trailing stalk cells, reducing the sensitivity of these cells to VEGF by altering VEGF receptor expression[85], and producing a non-uniform population of endothelial cells. This Delta-Notch system can be dysregulated in cancer[86]. Cell-cell adhesions (mediated by VE-Cadherin) can reduce VEGF-mediated EC migration[87]. Shear stress resulting from blood flow also regulates sprouting angiogenesis when blood flow is present[23]. Additionally, pericytes control angiogenesis and vessel stabilization by regulating EC proliferation and migration, along with contributing to formation of the vessel basement membrane[88, 89]. Pericytes express angiopoietin-1 (Ang1) and Ang2, which bind to Tie2 on endothelial cells[88]. Ang1 promotes vessel stabilization, while Ang2 destabilizes vessels. Endothelial cell-pericyte association is disrupted in many cancers, contributing to the formation of structurally and functionally abnormal vascular networks[44].

Due to the critical coordination of cells during sprouting angiogenesis, agent-based models (ABMs) are commonly used to study the evolution of sprouting in space and time. ABMs represent each cell individually, with specific logic rules dictating cell behavior, which may be time- or location-dependent[90, 91]. Rule-based ABMs can also be coupled with ODE- or PDE- based models, for instance of VEGF distribution in tissues[92, 93]. Such models can recapitulate directional sprouting in response to VEGF gradients, and capture emergent differences in sprout morphology under varying conditions[92]. Cellular Potts Models (CPM), also known as Glazier-Graner-Hogeweg (GGH) models, are lattice-based ABMs in which

each cell can evolve in shape, size, and interactions with other cells. As such, CPMs are used to study adhesion, cell elongation, and cell-cell signaling that alters EC behavior in angiogenesis and vasculogenesis[4, 6, 94, 95].

ABM cell behavior rules can be relatively simple, such as growth and movement based directly on experimental observations of dynamic cell behavior data in zebrafish[96]. ABM rules can also be more complex, basing cell behavior on detailed ligand-receptor dynamics and signaling, e.g. filopodia extension, migration, and proliferation, leading to tip and stalk cell behaviors, based on the Dll4, Notch, and VEGFR2 network by Bentley and colleagues[97, 98]. This model predicted that ECs in a nascent sprout can continuously compete for tip position, resulting in dynamic changes in tip and stalk cell specification, which has been experimentally validated[98]. This and other models and experimental data indicate that the Notch system may be an interesting potential therapeutic target[98–100]. In another study, the Glazier group has shown using CPMs that contact inhibition of cell proliferation or migration in response to extracellular stimuli can regulate vascular patterning[4]. Other ABMs have studied sprouting in response to combinations of VEGF and brain-derived neurotropic factor[101], and examined clean behavioral changes or knock-outs (e.g. tip and stalk cell proliferation and migration) that are not possible *in vivo* or even *in vitro*[102, 103], which is a key advantage of computational modeling as a tool to enhance drug design. Taking an alternate approach, a Boolean model links activation of combinations of VEGF receptors, integrins, and cadherins to cell behaviors such as migration and proliferation[104]. Together, these models improve our understanding of how combinations of extracellular cues regulate vascular remodeling, allowing for identification of new ways to modulate these processes *in vivo*.

As angiogenesis progresses, sprouts form lumens and anastomose onto existing vessels, facilitating blood flow and introducing these ECs to shear stress. Anastomosis requires the tip cell to become quiescent, a transition that has been studied by Bentley and colleagues using a Spring-Agent model, a type of ABM where each agent is a collection of smaller entities connected by spring-like tensions[105]. This allows for cell shape and cell-cell contacts to change, altering Notch signaling between cells. A multi-scale model of exercise response in skeletal muscle from the Popel group includes sprout formation, branching, and anastomosis in a single framework integrating blood flow, oxygen distribution, and VEGF transport (continuous processes) with cell behavior (discrete ABM)[106]. In this model, anastomoses occur when tip cells come within close proximity to other vessels, but molecular detail of anastomoses is not included. Simulations of tumor angiogenesis and blood flow incorporating shear stress-induced vessel branching[107], varying vessel morphology[108], and vessel pruning in response to therapy[109] suggest that vascular network morphology strongly influences delivery of both nutrients and chemotherapy drugs to tumors.

Some multi-scale models of vascular remodeling include other cell types, such as pericytes[110–113]. Pericytes must dissociate from vessels to permit sprouting, and their recruitment is required for vessel stabilization following remodeling. An ABM including pericyte recruitment in response to gradients of EC-secreted platelet-derived growth factor B (PDGF-B) and differentiation of interstitial cells into pericytes as a function of contact with

endothelial sprouts can predict the portion of capillary coverage by smooth muscle α-actin-positive pericytes[111]. A separate computational model captured vessel stabilization and destabilization in response to VEGF, PDGF, Ang1, and Ang2 by integrating modules for tumor growth, endothelial angiogenesis, and vessel stabilization (by pericytes)[112]. Vessel stabilization was predicted to result in slower tumor growth. This growth model predicted that anti-VEGF therapy is more effective when the portion of immature vessels is high, and that co-application of anti-VEGF and anti-Ang1 resulted in prolonged inhibition of tumor growth[112], in line with another model of metastatic ovarian cancer *in vivo*[113].

While many of these agent-based models consider only a small number of cells, understanding the initiation, extension, and anastomosis of angiogenic sprouts is essential to predicting structural and functional characteristics of developing vascular networks *in vivo*. Even on this small scale, differences can be observed between the behaviors of sprouts forming due to physiological and pathological angiogenesis. The ABMs presented here describe angiogenesis in healthy tissue[106], tumors[92, 94, 107–110, 112], the cornea[103, 113], and *in vitro* or developmental scenarios[4, 6, 98, 101], as well as studying sprouting in a generalized context[95, 97, 102, 104, 105, 111]. Some incorporate expression levels of cell surface receptors or protein concentrations[97, 98, 106], in order to understand how changes to these quantities alter sprout morphology in disease. Others integrate discrete models of angiogenesis with blood flow simulations[106, 108], increasing our understanding of the crosstalk between these differing regulatory mechanisms.

### 2.4 Microenvironment of the microvasculature: high-resolution molecular biology

Not only do ECs receive guidance cues from soluble factors and neighboring cells, but also from mechanical and chemical interactions with their microenvironment[114]. Spatial and temporal patterning of these cues is required for formation of functional vascular networks that effectively oxygenate the surrounding tissue[114]. The extracellular matrix provides a scaffold for tissues; changes in its stiffness are sensed by endothelial and other cells. Additionally, EC signaling is altered by integrin adhesion to ECM proteins[114]. ECs alter their microenvironment by secreting ECM proteins and proteases that degrade ECM components, clearing a path for vessel growth and remodeling. One family of proteases implicated in angiogenesis are the matrix metalloproteinases (MMPs), inhibitors of which are also expressed by ECs[115]. In addition to degrading ECM, proteases can also cleave VEGF, releasing previously immobilized VEGF into the interstitial fluid[114]. The microenvironment in solid tumors is much different than in normal tissue, with perturbed ECM organization and high vascular permeability[44, 116], while in peripheral artery disease the endothelial basement membranes are much thicker than in normoxic skeletal muscle[117]. Certain aspects of molecular regulation and cell-cell interactions can be studied *in vitro*, where detailed measurements are possible, but it is not feasible to exactly replicate the complete tissue microenvironment in which vascular remodeling occurs. Thus, multi-scale computational models are necessary to integrate the cues endothelial cells receive from their microenvironment and translate this information into predicted cellular behaviors.

A variety of modeling techniques have been used to study the influence of the microenvironment on vascular remodeling at higher spatial and temporal resolution than is

feasible experimentally. For example, a CPM (Cellular Potts Model, discussed in the previous section) of tumor angiogenesis can predict vascular branching and anastomosis of adjacent sprouts using rules based on molecular, cellular, and local tissue environment dynamics (VEGF gradients, proliferation rates, ECM composition) instead of observed cellular behavior[92]. In this model by the Jiang group, inhomogeneities in the extracellular environment were required to obtain realistic predictions. Additional study with this model demonstrated regulation by ECM fiber density and orientation of sprout extension and branching, suggesting that the ECM itself is a therapeutic target[95]. Other computational models, ranging from ABMs to multi-phase models, have demonstrated regulation of vascularization by pore size in porous scaffolds[118], collagen fiber orientation[119], and a combination of expression of soluble and matrix-bound growth factors, EC proliferation rate, and MMP activity[120].

In addition to the composition of the microenvironment, the local geometry surrounding an angiogenic sprout can significantly alter the availability of diffusible proteins to cell surface receptors. As such, the effect of distance between adjacent angiogeneic sprouts was studied in a 2D reaction-diffusion model by the Mac Gabhann group[121]. The model showed that decreased distance between two sprouts increased the probability that the sprouts would diverge. This study also demonstated that the VEGF-sequesting soluble VEGFR1 isoform, which is secreted by endothelial cells increases the gradient of VEGF-VEGFR2 along the length of sprouts[121]. These behaviors hold in extending the model to three-dimensional sprouts in tissues, and these models can provide molecular explanations for the observed behaviors of perturbed systems such as VEGFR1-knockouts[122]. These models are developed using high-resolution imaging of developing sprouts, enabling true image-based simulations that are specific to the different anatomical outcomes of the molecular perturbations.

Other computational models have focused on modification of the ECM due to endothelial secretion of proteases. Detailed models of the production, activation, and inhibition of several MMPs in the context of angiogenesis have been developed by the Popel group[123–125]. These models have been incorporated into larger 2D and 3D reaction-diffusion models of VEGF ligand-receptor binding and transport, and consider the release of HSPG-bound VEGF from the ECM via cleavage by proteases[126, 127]. It was shown that endothelial cells alone do not produce enough proteases to release a significant amount of VEGF, suggesting involvement of other neighboring cell types[126]. Additionally, simulation of the tissue distribution and gradient formation of HSPG-binding and non-HSPG-binding VEGF isoforms showed that isoform-specific degradation is necessary to match experimental measurements of VEGF localization, and is involved in vascular patterning[127]. These results are of particular relevance to tissue engineering, where the properties of the microenvironment can be tuned to promote proper vascular network formation. In addition to computatational modeling, high-throughput experiments and proteomic analysis have been used to understand the activity of MMPs and identify promising therapeutic targets[128–130]. The data generated by such studies can improve computational models of MMP activity in vascular remodeling and cancer[127, 131, 132].

### 2.5 Homeostasis requires coordination of multiple scales of regulation

While we have presented distinct levels of vascular regulation in this section, it is vital for understanding *in vivo* physiology to recall that all of these levels are interconnected. Diseases can alter any of these regulatory mechanisms, while drugs typically target gene expression and/or protein signaling networks within cells. Systems biology can aid in identifying the regulatory levels perturbed in specific disease states, which are not fully established for many diseases. After any perturbation (Fig. 2), the system can adapt using the outlined regulatory mechanisms, resulting in vascular remodeling and reaching a new homeostatic state. A specific example of a homeostatic cycle relevant to altered blood flow/oxygenation is shown in Fig. 3, along with the types of computational models that are used to study each process in the system. An example of multiscale modeling applied to skeletal muscle to simulate this entire homeostatic cycle will be discussed in **Section 3.3**. Other tissue-specific multiscale models with multiple cell types are emerging, including a study of oxygen and growth factors in healing bone defects[133–135]. While it is not computationally feasible to unite all of these modeling techniques in a detailed 3D model of the complete human body, we use a subset of these tools (application-dependent), the insights resulting from other models, and quantities that are experimentally measureable (*in vivo* and *in vitro*) to understand regulation of vascular remodeling at multiple scales, and how perturbations at any of these levels alters both local and system-wide behavior. This is turn will lead to improved ability to identify biomarkers and potential therapeutic targets.

## 3. MICROVASCULAR SYSTEMS PHARMACOLOGY

Vascular remodeling plays key roles, beneficial or detrimental, in many diseases[9]. Angiogenesis is a hallmark of cancer[136, 137], and ectopic vascularization drives retinopathies and other leading causes of blindness. In contrast, for diseases characterized by hypovascularization and/or ischemia, such as atherosclerosis, pre-eclampsia, Crohn's disease or hypertension, amelioration by the induction of angiogenesis or arteriogenesis continues to be an active area of therapeutic research. We focus here on cancer and peripheral artery disease as canonical diseases requiring anti-angiogenesis and pro-angiogenesis treatment, respectively.

Drugs, gene vectors, exercise, and other vascular-targeted therapeutic approaches can be studied using systems approaches. For example, the repeated lack of success in human clinical trials of proteins and genes encoding vascular-targeting growth factors suggests that scaling from mice and other pre-clinical models to humans is not trivial. The variability from person to person in responses to all drugs further complicates matters. Understanding the pharmacokinetics and pharmacodynamics of vascular-targeting agents is particularly difficult since the target cells for many of these – endothelial cells – have two active surfaces: one facing the blood stream where many of the drugs are delivered, and one facing the interstitial space of the tissue[138]. These two surfaces are not the same, and the effects of drugs at each surface are not the same.

Systems Pharmacology is crucial to improving the extremely low success rate in clinical trials generally. Clinical trials are very expensive, and using them we cannot try every target, drug combination, dose, or schedule. Systems Pharmacology enables us to virtually explore

the therapeutic space. Thus, we call on computational models to test and compare multiple drugs, drug combinations, doses, schedules and routes of administration. We can also go further than drugs to include non-drug therapeutics, including mechanical and electrical stimulation, exercise, or the implantation of engineered or transplanted cells and tissues. In this way we can efficiently eliminate therapies unlikely to be successful and focus on optimizing approaches predicted to show success for at least a subset of the patient population.

Clinical data – gene and protein expression, but also height, weight and other measurements – can be incorporated into well-designed models to build individualized simulations and populations of predictive patient models. On the other side, predictive models need to make clinically testable and measureable predictions, for example the dynamics of change to concentrations of key molecules in the blood. Only by validating such pharmacological models can we hope to make them useful in the clinic. These models can also help in prospective design of clinical trials by identifying key biomarkers, including complex or nonlinear biomarkers that would not be obvious from a linear analysis of the data.

### 3.1 Whole-body compartment models: pharmacokinetics and pharmacodynamics

As a consistent framework for the analysis of therapies – not just small molecule drugs and biologics, but also gene therapies, physiological changes, and tissue transplants – we must integrate the molecular and cellular understanding outlined in **Section 2** into a whole-body model that simulates the transport of key vascular regulatory proteins such as VEGF as well as their cellular targets. Clearly this cannot currently be done at the whole-body scale with the same level of three-dimensional anatomical detail and spatial resolution described in the models of **Section 2.4**; however, much of the anatomical specificity can be retained – for example, the multicellular nature of tissues; the heterogeneity of gene and protein expression between cell types; the volumes and surface areas associated with different cell types; the complex molecular interaction networks; and the dynamic nature of cells in responding to extracellular stimulus. By assuming each tissue compartment is well mixed, we can trade partial differential equations for ordinary differential equations[71, 139], significantly speeding up computation without losing much of the key biology regulating vascular remodeling.

In **Section 2**, we discussed the importance of blood in delivering oxygen to tissues and the importance of computational models in building a quantitative understanding of tissue physiology and pathology. The blood compartment also plays a central role in any systems biology perspective of disease and treatment, because blood-based measurements are the most common type of *in vivo* data available for validation of computational models. Accessibility, reproducibility, low invasiveness and the ability to do sequential measurements make blood biomarkers highly sought after. Simple one-component blood-based biomarkers can have clear population-level changes in pathology, but not be informative for an individual[140], suggesting that more complex biomarkers based on understanding of molecular mechanisms may be more informative. For example, a ratio of VEGF and sFlt1 protein levels in blood may be an important predictor of pre-eclampsia[141], better than either metric alone. Going beyond detection and diagnosis, prediction of blood-based biomarkers for disease progression and therapeutic response is an area of high interest

that opens the door to predictive, responsive and adaptive personalized medicine. Thus, understanding the relation between blood-based measurements (e.g. of soluble proteins) and disease state is an important goal that can be addressed using systems biology techniques.

### 3.2 Targeting angiogenesis in cancer: virtual clinical trials

Tumors can cause a perturbation to vascular homeostasis (Fig.2). At first, without vascular ingrowth, the tumor is oxygen limited. However, acquisition of pro-angiogenesis characteristics, such as the constitutive activation of HIF by oncogenic kRAS, can result in perfusion by new vessels. Because of the broken homeostatic cycle, hypervascularization and atypical vessels result – tortuous, inefficient and leaky. Tumor vascularization permits growth beyond the oxygen diffusion limit, and provides tumor cells a route for metastasis. Drugs developed to inhibit angiogenesis in cancer have targeted the key receptor tyrosine kinase pathways in vascular remodeling, including the VEGF receptors, EGF receptors (ErbB/HER) and FGF receptors on endothelial cells. These drugs include antibodies to ligands (e.g. bevacizumab) or to receptors (e.g. DC101) and tyrosine kinase inhibitors (e.g. sunitinib).

By building pharmacokinetic-pharmacodynamic (PK/PD) models of these growth factor-RTK systems, direct testing of multiple RTK-targeting drugs has been possible. These models can incorporate specific current drugs with known interactions and kinetics, but can also be used as a drug design tool by introducing molecules with different interactions. These models can give insight into whole classes of drugs and functions; for example, inhibiting receptor-receptor interactions has emerged from simulation of the VEGF/VEGFR system[61, 142–145] as a strategy potentially superior to ligand targeting[61]. This is being borne out in recent experimental results for drugs targeting receptor dimerization[146]. More recently, the tendency of tumors to favor the expression of specific VEGF isoforms was identified using computational simulation to be a critical vulnerability and improve the predicted efficacy of anti-tumor VEGF-targeting[144]. The predicted impact of isoform-specific anti-VEGF agents are not as might be expected based on our understanding of physiological angiogenesis, in part because the regulation of isoforms is very different in tumors[147].

An alternate model of VEGFR pharmacodynamics goes beyond the ligand-receptor interactions by incorporating VEGFR2's downstream signaling pathways[148]. By doing this, the Birtwistle and Gallo groups were able to run sensitivity analyses of dosing for multiple drugs targeting VEGF, VEGF receptors and downstream signal molecules such as PLCγ. They then used optimization algorithms to define potential multidrug regimens with different dosing and scheduling[148].

Validation of pharmacological models is crucial to developing helpful predictive simulations. For models of human pharmacology, the detail and complexity of the models results in many outputs that are not easily measurable, e.g. cell-type-specific activation of multiple receptor families, but also several that are. In particular, the models can predict the effect of multiple perturbations in different cells in different tissues on key proteins in the blood. For example, a multi-compartment PK/PD model of VEGF in humans was used to investigate dynamic changes in the tumor and in the blood following treatment with

systemic infusion bevacizumab (anti-VEGF antibody). Counterintuitively, and without any fitting of data, the model predicted that the concentration of VEGF in blood would increase following anti-VEGF treatment[145]; this surprising effect has indeed been clinically observed[149–151]. Because of the highly detailed and mechanistic nature of the model, we could go further and determine that this emergent property resulted from a shuttling mechanism of the VEGF-antibody complex[145]. Such mechanistic hypothesis testing can result in strong and actionable therapeutic predictions.

Another key requirement of models – to be populated with high-quality, detailed experimental data – becomes a benefit of taking an integrated (experimental and computational) systems approach. Models can help us to identify which experimental measurements (target, type, location, spatial resolution and temporal resolution) are the most important or informative. For example, pharmacological models have identified that cell-specific receptor expression plays an important role in the response to therapy – many RTKs are expressed on multiple cell types and not just on the target cell type[152] and the potential for synergistic or antagonistic side effects is clear. Model-based quantification of these multi-cellular (and multi-tissue) effects is clearly important to prediction of therapeutic outcome. Based on simulations, delivery of a VEGF-neutralizing agent can result in available VEGF in the tumor going either up or down depending on the variability in both ligand and receptor expression[142, 144]; even the difference between the apical and basolateral expression of VEGF receptors was predicted to play a major role in pharmacodynamics[153] and this prediction of a systems biology model is now being borne out[138].

### 3.3 Promoting vascularization in peripheral artery disease: from rodent to human

While therapies targeting hypervascularity in cancer and age-related macular degeneration have come to market, no pro-angiogenesis therapeutic agents have been approved. Indeed, multiple trials have failed[56, 154, 155], including proteins or gene therapy targeting VEGF, HIF-1 or FGF. These failures occurred despite successes in pre-clinical animal models of ischemic disease. Thus, there is an urgent need for systems biology techniques to help predict which treatments would be successful, providing a better bridge from pre-clinical to human clinical trials.

To study the *in vivo* pharmacodynamics of angiogenesis-targeting treatments, we have developed two types of multi-scale models. First, a fully three-dimensional model, that uses image-based anatomical information to simulate a portion of tissue at micron resolution – for example, skeletal muscle (Fig.4A). While simulations using this model are confined to a particular volume of tissue, the pharmacodynamics of key treatments can still be tested – for example: local effects of gene delivery, which will alter the cell-specific expression rates in the model; or cell-based therapy, in which augmented stem cells can differentiate and integrate into the tissue; or exercise, which will impact gene expression but also blood flow and oxygen demand[68, 69, 156, 157]. These three-dimensional simulations identified key drivers of the VEGF concentration in the tissue as well as of VEGFR activation. Even at rest, without disease or external perturbation, there is heterogeneity in oxygen, VEGF expression, and VEGF and VEGFR concentration gradients. This was further studied using

a more detailed anatomical model that included realistic muscle fiber type distributions[35]. The expression of VEGF receptors, and thus the location of the blood vessels, was identified as the key driver of VEGF gradients (which are thought to provide chemotactic guidance to nascent sprouts). We noted that exercise, which is encouraged therapeutically for PAD patients but is often difficult especially in more severe disease, results in up-regulation of both VEGF ligands and VEGF receptors. We were then able to identify using our models that therapeutics delivering only ligands are less effective at increasing the concentration gradients in tissues, and can induce these increases for a shorter time, than receptor expression changes. This, then, provides a possible path forward in developing the next generation of PAD therapeutics. Based on these models, we added an agent-based model of cell behavior to 'complete the circle' (Fig.3) and enable the simulation of chronic disease and treatment, or repeated bouts of exercise training[93].

Building a whole-body three-dimensional model with the resolution needed to deal with the molecular gradients described above is not currently feasible. Instead, a second kind of model is needed – a compartmental PK/PD model[158], similar to that described in the previous section for cancer, but now with a target 'disease' tissue of the ischemic calf muscle (Fig.4B). Although concentration gradients cannot now be simulated at this scale, we can test systemic organism-wide perturbations, such as sleep/wake and exercise cycles, which impact lymphatic flow as well as molecular expression[70, 159] (Fig.4B), the impact of therapeutics on non-target not-diseased normal tissues, and the intravascular delivery of therapeutic molecules.

These two model types – 3D high-resolution models of tissue and the compartmental PK/PD models – can be directly compared because the interstitial concentrations in the compartments will be the same as the average concentrations adjacent to the interstitial surface of VEGFR-expressing ECs; the average VEGFR activation in the 3D model will be the same as the compartment-level VEGFR activation in endothelial cells.

Lastly, we note that a key issue in the treatment of peripheral artery disease is the failure in humans of treatments that work in rodents. This is a common problem and one for which systems biology is well suited. The parallel development of mouse-specific and human-specific computational models, with a common framework and species-specific parameters, will enable the translation of findings in one to predictions of successful approaches in the other.

## 4. CHALLENGES AND FUTURE DIRECTIONS

A wide variety of computational and experimental techniques have been harnessed to expand our knowledge of microvascular function in health and disease. Computational models are invaluable in their ability to integrate multiple experimental results into a single, often mechanistically-based framework. Progress has been made in integrating across multiple model types, biological regulation mechanisms, and geometric scales to provide a systems-level, dynamic view of the microvasculature and of its remodeling processes. And yet much remains to be done to meet the challenge of making these models, and our

resultant understanding of this complex dynamic system, capable of bridging insights from the lab to the clinic.

Areas of potential growth include the development of species-specific and personalized models. Mouse-specific and human-specific models, parameterized with species-specific experimental data, can be used side-by-side to assist in successful translation from pre-clinical to clinical trials. Patient-specific models can incorporate not only individualized pharmacokinetic parameters but also the high variability in gene and protein expression that greatly affect pharmacodynamics. Such models can advance identification of biomarkers for specific subpopulations, and identify specific therapeutic strategies as being effective (or ineffective) for each group[160].

It is crucial, as increasingly complex computational models are developed, to validate model outputs against quantities that are measurable *in vivo*, while leveraging non-measurable model outputs to predict changes in cellular signaling and behavior that may be important for disease prognosis and response to therapy. In parallel with continued model development, systematic collection of quantitative experimental measurements to characterize vascular growth and remodeling in healthy and diseased tissue, both before and after treatment, is critical to develop a sufficient mechanistic understanding of microvascular dynamics to provide meaningful clinical decision support. And as these models and experimental data are produced, it is essential to perform failure analysis – to probe the molecular mechanisms behind the failure of unsuccessful pro-angiogenic drugs. There is so much to learn from previous preclinical and clinical trials that can inform future therapeutic design.

There is also a need for further study of the less well-understood forms of vascular remodeling, such as arteriogenesis and capillary arterialization. In addition, more must be done to understand the layered and complex effects on vascular remodeling and therapeutics of key co-morbidities such as diabetes and hypertension. In the clinic, patient presentation is rarely single-factor, and a systems approach to multi-disease interactions could greatly improve outcomes.

While there remain many challenges to be met in microvascular systems biology, the progress of recent years highlights the value of systems computational and experimental approaches, and promises advances in clinical outcomes in the years to come.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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The vascular network carries blood throughout the body, dynamically adjusting to maintain tissue oxygenation. The network and its constituent cells adapt in response to both acute and chronic stimuli. Controlling these key physiological and pathological processes is of great interest in many diseases. A systems biology approach is essential to unite our understanding of vascular physiology across the molecular, cellular, and tissue scales. Multiscale computational models can provide the bridge necessary to more effectively translate *in vitro* results to *in vivo* systems, and to translate pre-clinical animal models of disease and treatment to human therapy and clinical trials in the future. We present several examples of computational models that highlight the potential of systems biology approaches to generate novel insight into *in vivo* vascular biology.

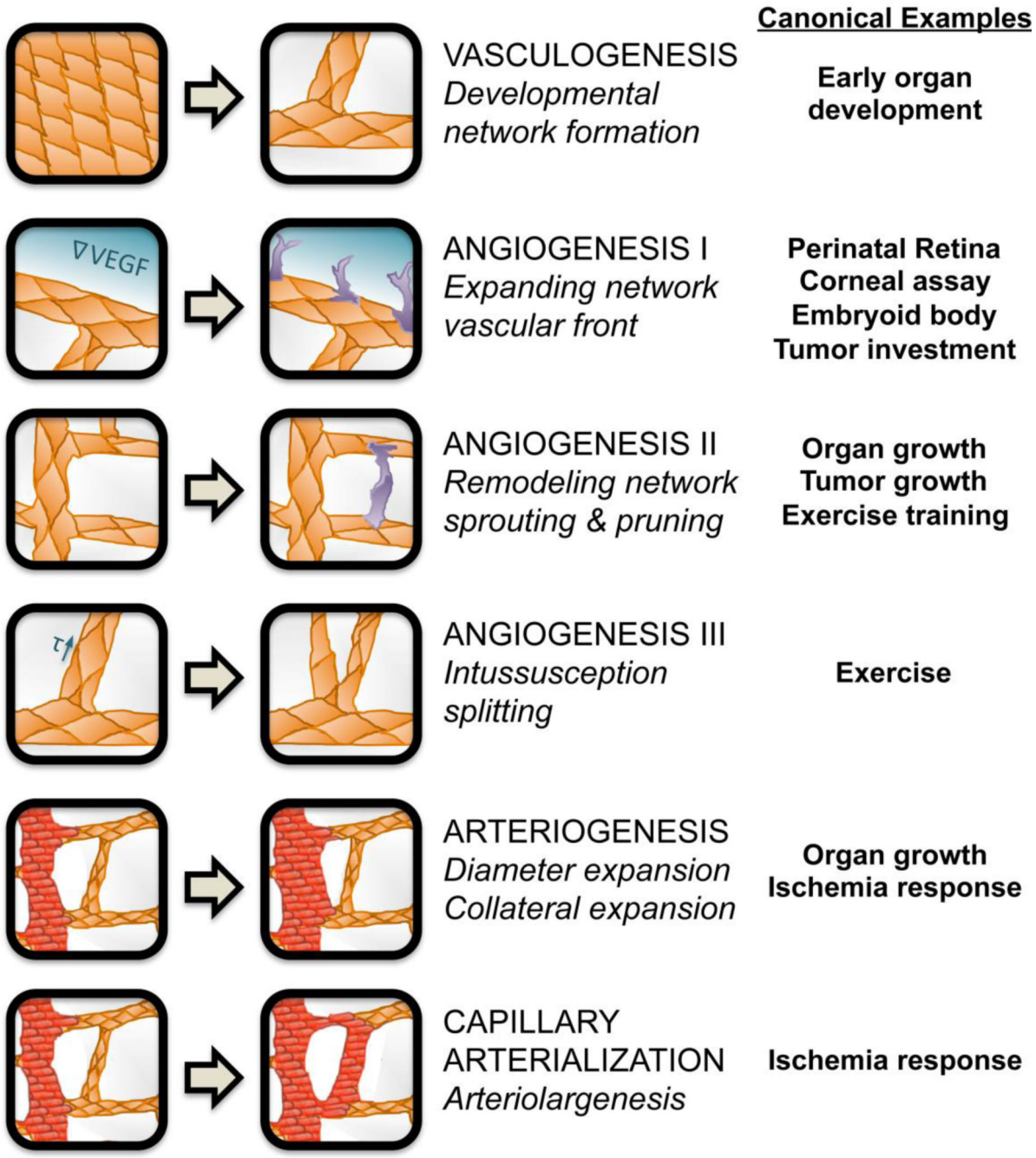


**Figure 1. Vascular development and remodeling processes**

The six distinct types of *in vivo* blood vessel formation or remodeling, described in the text, are prevalent in different tissues and situations. Both the emergence and the dynamic adaptation of a functional hierarchical vascular system depend on the coordinated regulation of all these processes. **Vasculogenesis** results in *de novo* vessel formation, which is critical for development, while **angiogenesis** involves expansion of the existing network via sprouting or vessel splitting, and is required for network expansion. **Arteriogenesis** and **capillary arterialization** allow for remodeling of the vascular network in response to

stressors such as ischemia, to alter blood flow within existing tissues. Examples of *in vivo* situations in which each process is particularly relevant are given.

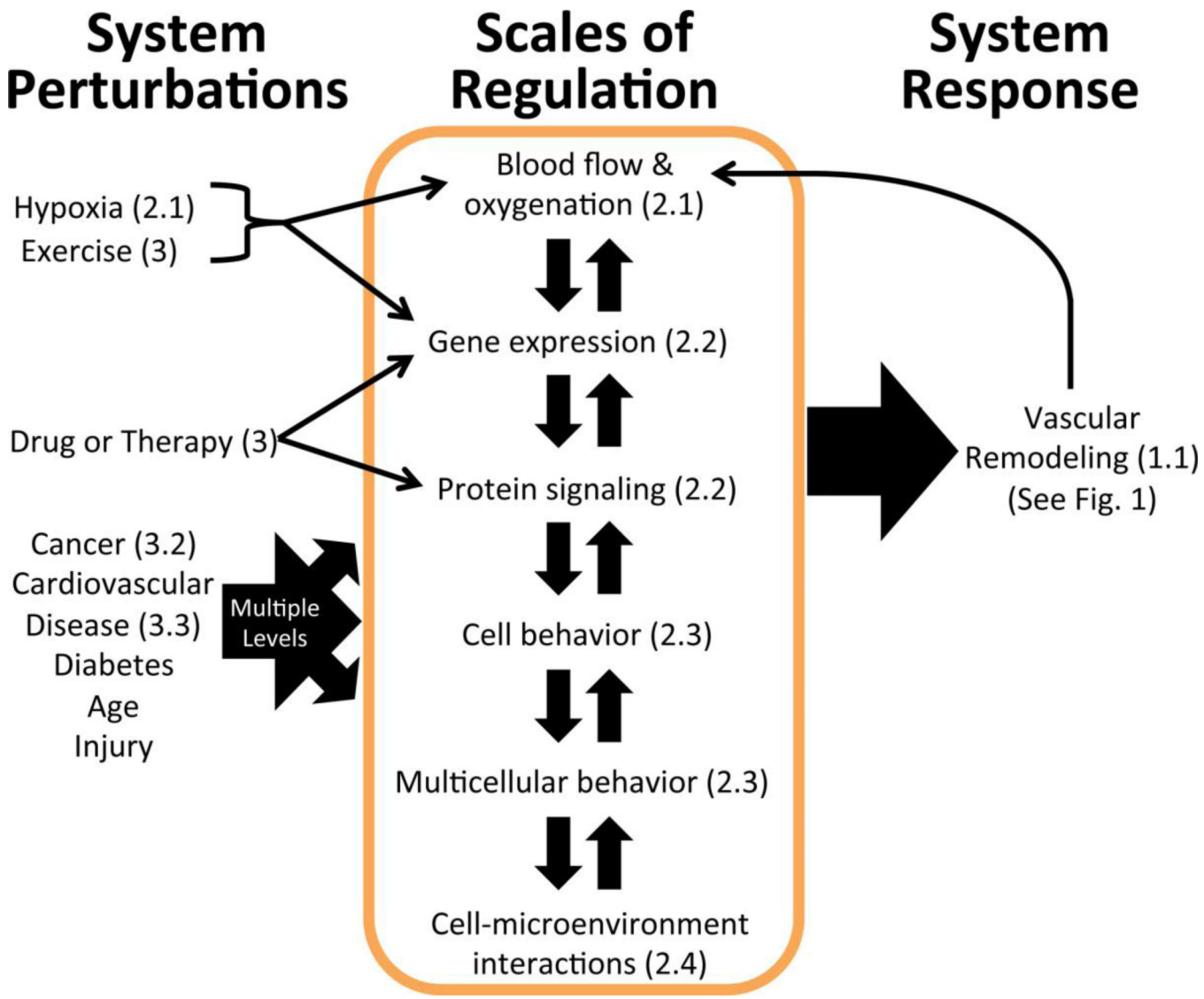


**Figure 2. Vascular remodeling is a system-wide response to various perturbations at different scales**

Vascular homeostasis can be perturbed by disease, therapy, exercise, injury, or aging (left column). While some of the perturbations introduced by disease are relatively well-characterized (cancer, cardiovascular disease & hypoxia), others represent opportunities for future systems biology research (diabetes, age-related changes). These perturbations directly alter one or more of the scales regulating the vascular system (center box, discussed in Sections 2.1–2.4 as indicated), and propagate due to the connectedness of the system, inducing indirect changes at the other levels of regulation as well. As the vascular system adapts to the perturbation via remodeling (See Fig. 1), a new homeostasis is established (right column). This new homeostatic state may have different blood flow and gene expression than the pre-perturbation system, depending on the effectiveness of the physiological or therapy-induced remodeling. While perturbation/dysfunction can occur at any of the levels, most therapies target molecular regulation mechanisms (Section 2.2).

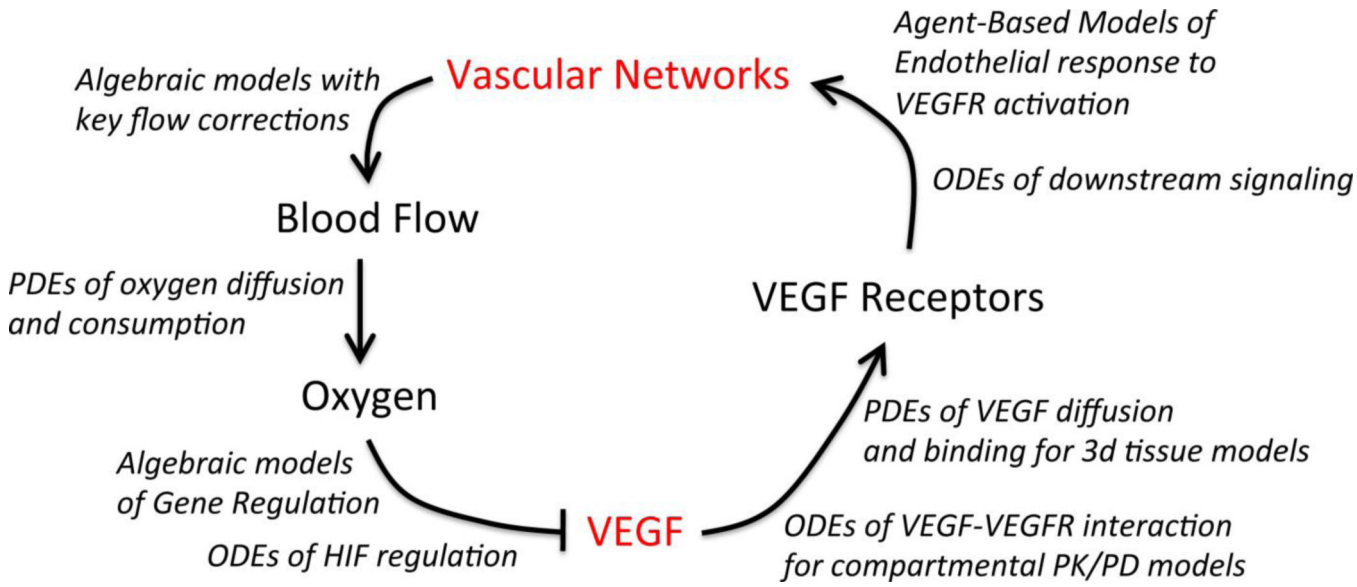


**Figure 3. An example of vascular homeostasis and regulation by VEGF**

The many different computational model types employed to simulate the flow of information through the integrated multi-scale physiological models are indicated in italics. In general, models of *in vivo* pathology incorporate key elements of tissue physiology: vascular network geometry, blood flow, and/or oxygen distribution. Detailed models of molecular and cellular regulation, for example of the VEGF family, are often constructed and validated with *in vitro* experimental data, and then integrated into *in vivo* models and coupled to the other scales of regulation (Fig. 2) to predict the vascular remodeling and other physiological changes resulting from molecular perturbations (such as therapeutics). In diseases such as cancer, the homeostatic regulatory mechanisms can become non-functional or function in altered ways, leading to different vessel morphology than observed under physiological conditions.

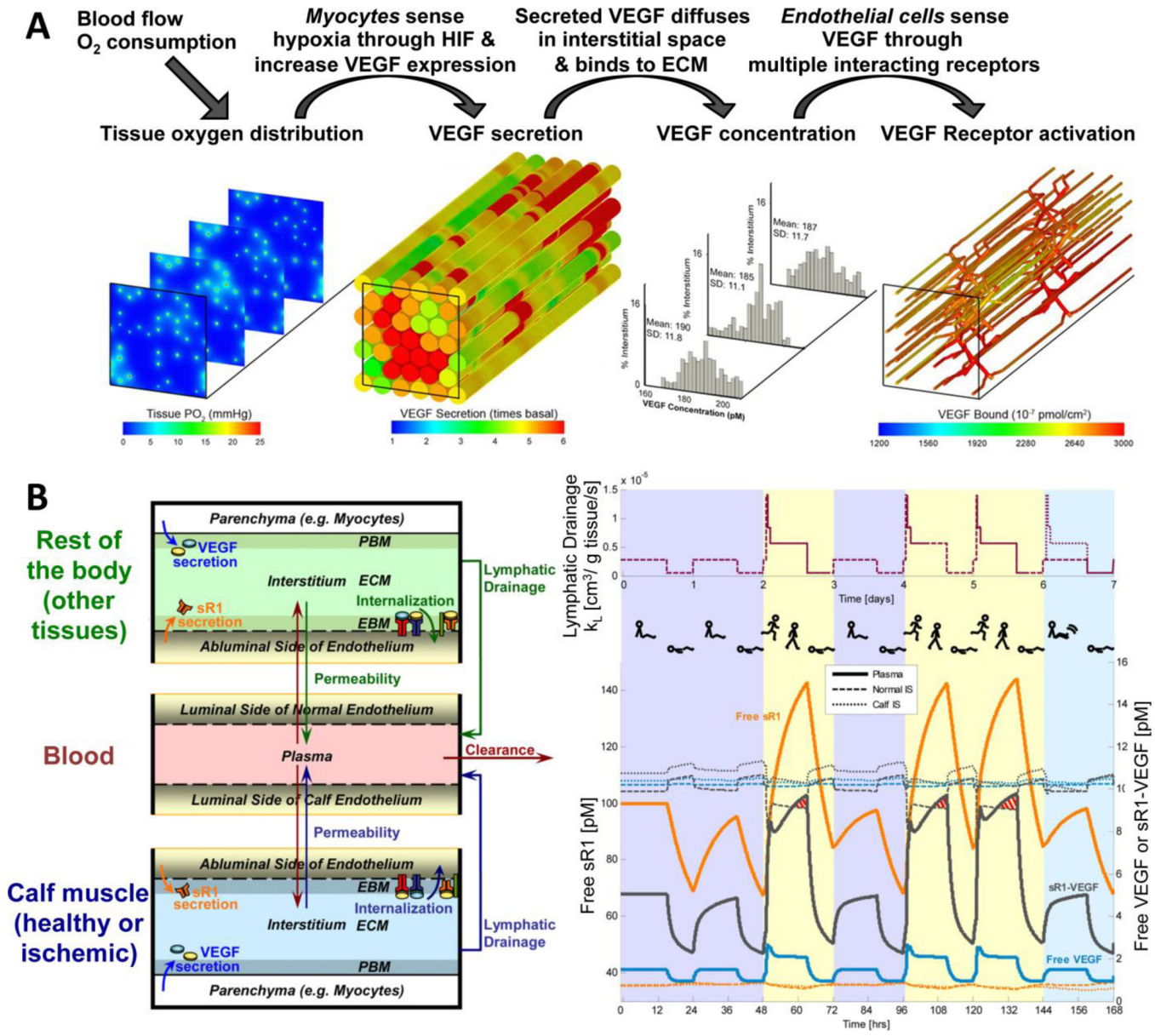


**Figure 4. Multi-scale models of microvascular physiology and pathology *in vivo*. A, Three-dimensional multi-scale model of vascular regulation in skeletal muscle *in vivo***

By integrating multiple model types (Fig.3), we can simulate the links from three-dimensional tissue anatomy and heterogeneity to blood flow, to oxygen distribution, to hypoxia-dependent VEGF secretion by parenchymal cells, to VEGF diffusion, to ligation of VEGF receptors on endothelial cells. The output is heterogeneous VEGF receptor activation across the vasculature, which can then be coupled to cell behavior models such as ABMs[93, 106] to complete the homeostatic cycle and remodel the vascular network. This integrated model has been used to study peripheral ischemia disease and to test potential treatments. Simulation results figures adapted from[157]. **B, Multi-compartment PK/PD model of the VEGF family**. This model has multiple compartments, including calf muscle to enable studying the effects of PAD which results in significant pathological changes to

that muscle. The model predicts the distribution of VEGF and soluble VEGFR1 and VEGF receptor activation throughout the body, including the blood concentrations of the diffusible proteins. The compartments of the PK/PD model can communicate via physiological processes such as vessel wall permeability and lymphatic drainage. An example application of the PK/PD model is also shown, a simulation of the dynamic effects of diurnal changes in lymphatic drainage (as a result of changes in posture and activity) on plasma soluble VEGFR1 and VEGF levels in a healthy patient. Purple background represents bed rest days, yellow represents active days, and aqua shows calf rest days. Models of this form allow for prediction of tissue VEGF concentrations, and net flows of VEGF between multiple tissues and the blood, and are also druggable – small molecule, protein and gene therapies can be added, as can therapeutic alterations to exercise scheduling. Schematic and simulation results figure adapted from[159].