Integration of experimental and computational approaches to sprouting angiogenesis

Shayn M. Peirce[a], *Feilim Mac Gabhann*[b,c], *and Victoria L. Bautch*[d,e,f]

Purpose of review
We summarize recent experimental and computational studies that investigate molecular and cellular mechanisms of sprouting angiogenesis. We discuss how experimental tools have unveiled new opportunities for computational modeling by providing detailed phenomenological descriptions and conceptual models of cell-level behaviors underpinned by high-quality molecular data. Using recent examples, we show how new understanding results from bridging computational and experimental approaches.

Recent findings
Experimental data extends beyond the tip cell vs. stalk cell paradigm, and involves numerous molecular inputs such as vascular endothelial growth factor and Notch. This data is being used to generate and validate computational models, which can then be used to predict the results of hypothetical experiments that are difficult to perform in the laboratory, and to generate new hypotheses that account for system-wide interactions. As a result of this integration, descriptions of critical gradients of growth factor–receptor complexes have been generated, and new modulators of cell behavior have been described.

Summary
We suggest that the recent emphasis on the different stages of sprouting angiogenesis, and integration of experimental and computational approaches, should provide a way to manage the complexity of this process and help identify new regulatory paradigms and therapeutic targets.

Keywords
computational models, experimental models, sprouting angiogenesis, stages of angiogenesis

INTRODUCTION

Blood vessel formation is critical to the development of all vertebrates, and co-option or dysfunction of blood vessels in diseases such as atherosclerosis and cancer is a major cause of mortality in humans. Thus, the molecular and cellular mechanisms that contribute to formation of a vascular network are potential therapeutic targets, and our understanding of these regulatory inputs has increased exponentially in the last several years. Indeed, we now have clinically approved therapies (e.g. Lucentis; Genentech, USA) that are widely prescribed in certain disease settings, such as diabetic retinopathy, to effectively modulate key molecular signals, such as the vascular endothelial growth factor (VEGF) signaling pathway. We understand that differential responses to inputs by the endothelial cells that comprise the nascent vessels, so that some cells migrate, whereas others divide, is important for the expansion of blood vessel networks via sprouting angiogenesis. We know many of the molecular pathways that contribute information for proper vessel sprouting, and we know that some of them, such as VEGF and Notch, integrate with each other to establish endothelial heterogeneity and appropriate responses.

We have also come to realize that blood vessel sprouting is a complex process, and a complete understanding of its regulation will require knowledge of which inputs are critical, and when in time

[a]Department of Biomedical Engineering, University of Virginia, Charlottesville, Virginia, [b]Department of Biomedical Engineering, [c]Institute for Computational Medicine, Johns Hopkins University, Baltimore Maryland, [d]Department of Biology, [e]McAllister Heart Institute and [f]Lineberger Comprehensive Cancer Center, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Correspondence to Victoria L. Bautch, PhD, Department of Biology, CB#3280, The University of North Carolina, Chapel Hill, NC 27599, USA. Tel: +1 919 966 6797; fax: +1 919 962 8472; e-mail: bautch@med.unc.edu

Curr Opin Hematol 2012, 19:184–191
DOI:10.1097/MOH.0b013e3283523ea6

KEY POINTS

- Experimental tools are being developed that harness high-resolution microscopy and precise molecular manipulations in order to produce detailed descriptions of in-vitro and in-vivo sprouting angiogenesis.
- The development of new theoretical approaches, in parallel, provides quantitative and systems-level information about sprouting angiogenesis that is difficult to obtain and integrate without the help of computers.
- The next frontier is integration of experimental and computational approaches to provide a sophisticated understanding of the complex process of sprouting angiogenesis.

and space they are important. In addition to temporal and spatial information, quantitative aspects and integration of different signaling pathways are also important parameters for a complete picture of vessel sprouting and network formation. Sophisticated experimental tools are increasingly available to tackle these issues. For example, it is now possible, using recombination-based genetic manipulations, to regulate the expression of exogenous genes or to selectively remove gene function in both time and space *in vivo* in mouse and to some extent in zebrafish, and this ability has been essential to developing the paradigms described below for blood vessel sprouting. We also have achieved high-resolution imaging in zebrafish embryos and mouse embryonic tissues and derivatives (i.e., embryonic stem cell differentiation) to provide spatial information, and adaptation of these imaging modalities for live imaging has begun to provide temporal information as well.

Complementary to experimental approaches is the recent development of theoretical computational models to better understand the system-level principles (Fig. 1) that underpin blood vessel formation; these models can also be used to generate testable hypotheses. Theoretical models can quantitatively compute what is often difficult or impossible to measure experimentally, account for the combinations and permutations of signaling networks that give rise to observable phenotypes, and offer the researcher an inexpensive 'playground' upon which to test hypotheses that may be challenging to test experimentally. Recent theoretical models of angiogenesis have offered some new and different insights, which have suggested new lines of basic research and opened up new possibilities for therapy design.

This short review will provide a brief description of sprouting angiogenesis, and suggest that dividing the process into distinct stages is helpful both experimentally and computationally. We summarize recent computational models of sprouting angiogenesis and vessel network formation, including new work from our groups. Finally, we will discuss some of the important challenges and critical questions going forward.

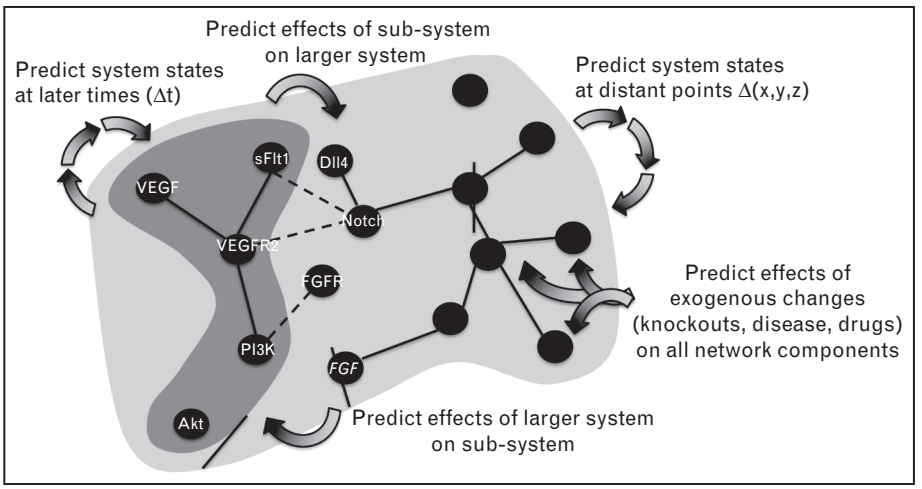

FIGURE 1. A systems-level perspective to studying angiogenesis. The typical reductionist approach enables close examination of a few interacting signals (dark gray) within a submodule of the system, and linkages between signaling modules (dashed lines) within entire networks are often inferred or hypothesized. In contrast, computational models can provide systems-level perspectives through the prism of biological complexity, contextualizing the comprehensive network of signaling modules that give rise to system behaviors and how they change over time to produce different states (light gray). Dll4, Delta-like 4; FGF, fibroblast growth factor; FGFR, FGF receptor; PI3K, phosphoinositide-3-kinase; sFlt1, soluble FMS-like tyrosine kinase-1; VEGF, vascular endothelial growth factor; VEGFR2, VEGF receptor 2.

SPROUTING ANGIOGENESIS

Endothelial cells differentiate from mesoderm and form nascent vessels via a process called vasculogenesis; once a primitive vessel is formed, further expansion and interconnection with other vessels proceeds primarily via sprouting angiogenesis, the coordinated outward migration of groups of endothelial cells called sprouts [1]. This migration is accompanied by endothelial cell division to allow for expansion of the network. The concept that endothelial cells have different responses to proangiogenic cues such as VEGF-A is exemplified by the understanding that sprouts contain both tip cells and stalk cells [2]. Tip cells initially respond to VEGF-A by migrating outward from the parent vessel, whereas stalk cells behind the leading tip cell migrate only passively and divide in response to VEGF-A. Subsequent studies showed that Notch signaling within the sprout is important for allocation of proper numbers of tip cells and stalk cells [3–5]. This concept has been expanded to include endothelial cells that are neither tip nor stalk, and these cells are called phalanx cells or lateral base cells if next to the emerging sprout [6,7]. Pericytes are also associated with nascent vessels and affect endothelial cell responses to signals in poorly understood ways. Overall, pericyte coverage of a vessel is associated with quiescence, analogous to the phalanx phenotype; however, pericytes also interact with stalk cells and even tip cells during sprouting [8▪]. Recent work has shown regulation of vessel sprouting via microRNAs in both mouse and zebrafish [9,10▪,11▪▪]. Angiogenic sprouting also appears to be fine-tuned by modifications to Notch signaling via the deacetylase Sirtuin 1 [12▪].

Sprouting is accompanied by lumen formation that occurs behind the migrating tip cell, and in many sprouting vascular beds lumenogenesis is considered to be a property of stalk cells but not tip cells [13]. Lumen formation allows for blood flow that often initiates the process of remodeling the primitive vessel network that forms via angiogenesis. Although poorly understood, recent work is consistent with a cord-hollowing mechanism of vascular lumen formation, whereby endothelial cell–cell borders set up an apical domain, and subsequent shape changes lead to lumen formation [14,15,16▪]. The final step in sprouting is the fusion of a sprout with another sprout or vessel to form a new connection. Although this process is also not well understood, a recent study suggests that in some cases macrophages may act as chaperones to organize fusion events [17▪].

The recent recognition of sprouting angiogenesis as a multistaged process (Fig. 2) has been facilitated by in-vitro and in-vivo assays that are

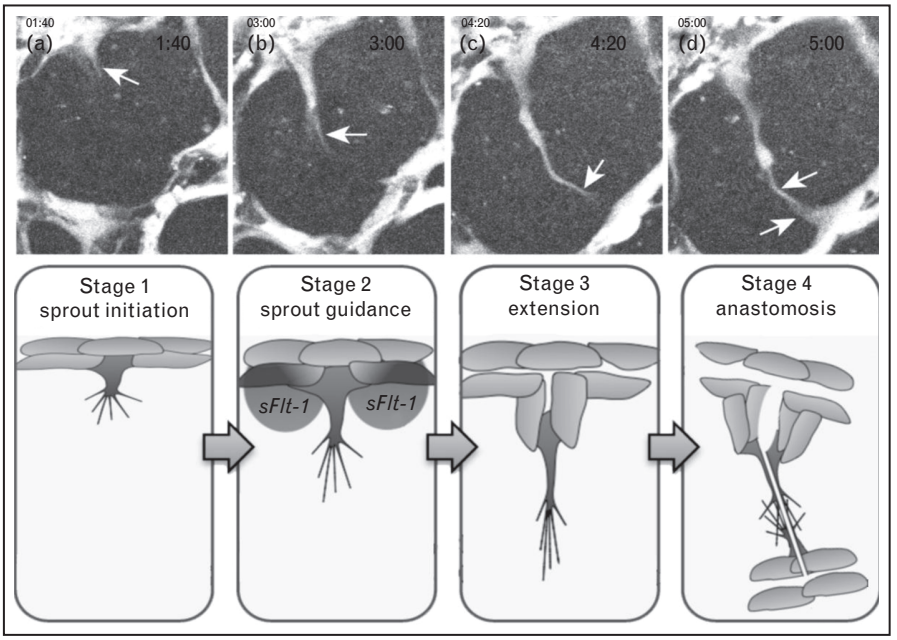

FIGURE 2. Stages of angiogenesis. Time lapse images of embryonic stem cell-derived vessels demonstrating the four 'stages' of endothelial sprouting angiogenesis described in the text: (a) sprout initiation, (b) sprout guidance (e.g., through a channel created by sFlt-1 binding VEGF around adjacent stalk cells), (c) sprout extension, and (d) sprout anastomosis. Endothelial cells (white) of mouse embryonic stem cell-derived vessels have been genetically modified to express eGFP and visualized using confocal microscopy. Time stamps = time elapsed (h) since start of run. (Images courtesy of Dr John Chappell, Ph.D, University of North Carolina) [18▪▪,19▪▪,20▪,21▪▪].

amenable to high-resolution fixed and live imaging with single-cell resolution. These models can also be perturbed genetically and/or pharmacologically, and thus have provided detailed descriptions of molecular pathways that are important to the overall process of angiogenesis in the given model system. However, these pathways have not yet been scrutinized for their role within each stage of this complex process. *In vivo*, the postnatal mouse retina and the zebrafish embryo have been crucial to elucidating angiogenic mechanisms. This has been both advantageous and problematic: many studies use similar models and thus can be compared; however, much experimental data that supports our 'generic' conceptual models of vessel sprouting comes from a few fairly specialized vessel beds.

For example, the vasculature of the mammalian retina forms outwardly from the optic nerve during the first postnatal week [22]. This vessel network is accessible to physical manipulation via intraocular injection and genetic perturbation, and it is amenable to high-resolution imaging. Similarly, the zebrafish embryo has been spectacularly successful as a model for live imaging of sprouting vessels, and it is also tractable for forward genetic screens that have yielded novel molecular players in sprouting [23]. In-vitro models of sprouting angiogenesis are many and varied, and most are amenable to live imaging. Among the most robust is a sprouting model of endothelial cells in which human umbilical vein endothelial cell-coated beads in a fibrin matrix sprout and form lumenized vessels [24]. Another model involves a programmed differentiation of mouse embryonic stem cells to form lumenized vessels over the course of a week [25]. In a variant of this model, partially differentiated embryonic stem cell-derived embryoid bodies are embedded in collagen with added VEGF-A, and developing vessels sprout outward into the matrix [26].

These and other recently established experimental models have provided a rich trove of information about cell behaviors and their molecular regulation during blood vessel sprouting, and more simple experimental setups (i.e., microfluidics chambers) have provided quantitative and spatial information about signaling cues [27,28]. It is apparent, however, that a full understanding of how angiogenic sprouting and vessel network formation are regulated will require the synthesis and integration of data from multiple perturbations and different model systems. It will be important to understand which signaling paradigms are consistent across (or unique to) specific tissues and vascular beds, to determine how capillary sprouting differs across species and between in-vitro and in-vivo models, and to explore network-level relationships between relevant molecular signaling pathways. An additional goal is to identify new drug targets that may be concealed by redundant pathways or genetic compensation. With these aims in mind, a number of useful theoretical computational models (Fig. 3) have recently emerged that provide a more quantitative and synthesized perspective on the 'system' of sprouting angiogenesis.

MODELING SPROUTING ANGIOGENESIS

Although many theoretical models of angiogenesis are rooted in mathematics and employ numerical techniques to describe physical phenomena according to fundamental laws (e.g. thermodynamics, conservation of mass, etc.), they are almost always grounded in empirical data. Indeed, the recent explosion in available data due to the emergence of high-throughput analyses, precise molecular assays, and a wide range of experimental models has greatly facilitated the ability to determine both the conceptual models and detailed parameters needed for constructing theoretical models of angiogenesis. This, coupled with faster computer processing speeds, parallel computing, and user-friendly programming interfaces, has greatly facilitated the generation of computational models of angiogenesis in recent years.

There are as many ways to theoretically model biological processes as there are experimental tools. It is now possible to integrate computational models and experimental data to better understand blood vessel sprouting, and one approach that is currently in wide use is very simplistically stated as follows: build the computational model based on experimentally derived knowledge (e.g. obtain model parameters directly from empirical data), validate the model by comparing its predictions to independent experimental parameters (i.e., not ones that were used to construct the model), challenge the model to predict the outcome of a new and different set of simulated conditions (i.e., test a novel hypothesis and/or generate new hypotheses).

There are many excellent, recent studies in the literature that have used this approach for developing and employing computational models to investigate mechanisms of capillary sprouting angiogenesis (Fig. 3). Recent models can be loosely binned into those that have studied the environmental milieu with molecular detail (e.g. growth factor concentrations and concentrations of ligand/receptors) and those that have focused on cell behaviors and cell–cell communication.

Theoretical ligand–receptor kinetic binding models have yielded important quantitative insights

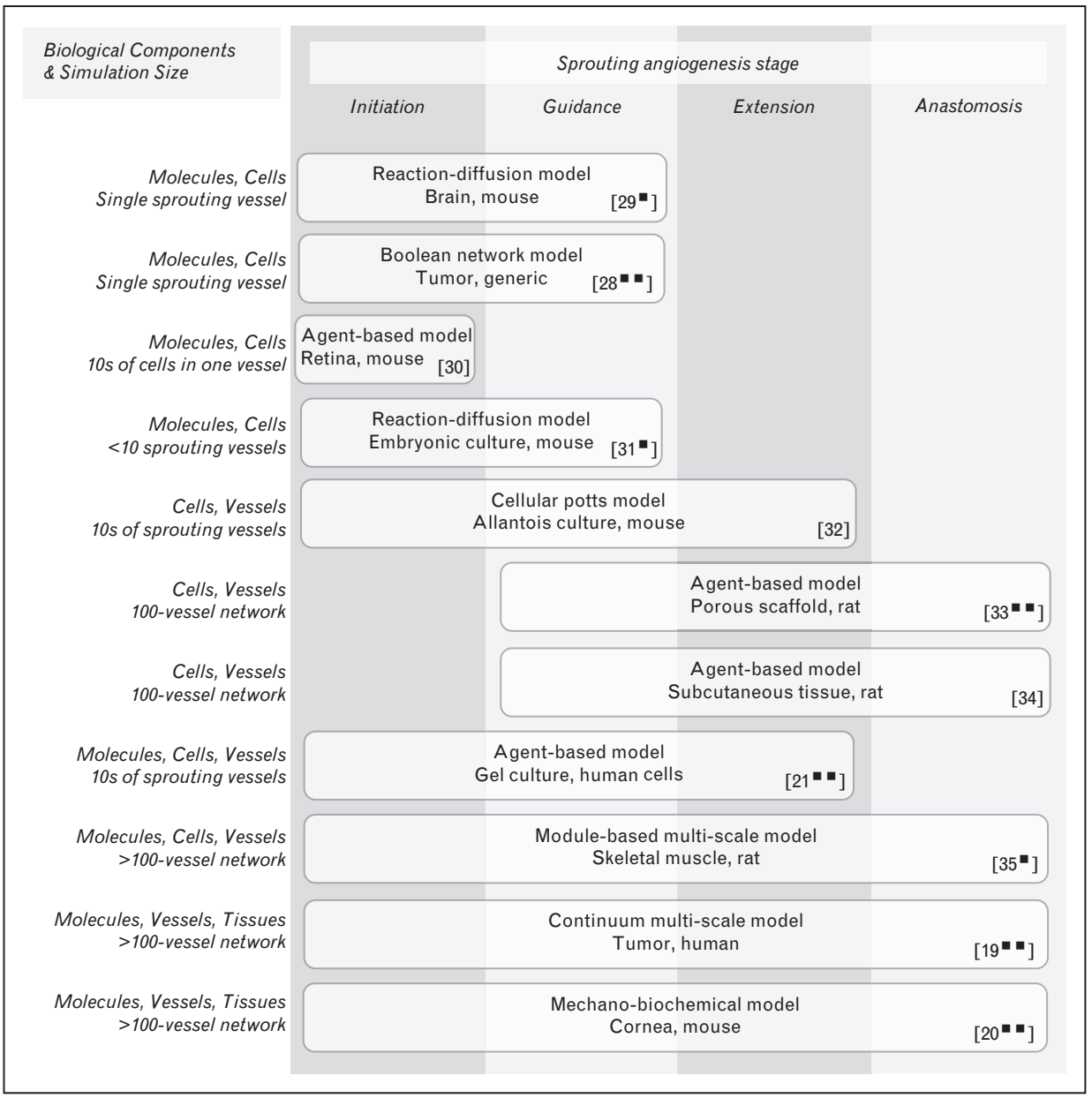

FIGURE 3. Summary of computational models of angiogenesis that span different stages. Each of the multiscale computational models described in the text is of a particular type – primarily agent-based (cell-centric) or reaction-diffusion (molecule-centric). The models can be classified according to the stages of sprouting angiogenesis that they simulate, as well as the biological components (molecules, cells, vessels, parenchymal cells) that they include. Each model also has a different simulation size, ranging from a single sprouting vessel to a complex multivessel network. In each case, the citation number refers to the reference list in the main text.

about VEGF signaling within tumors [36▪] and muscle [37▪], primarily because of their ability to predict what cannot be measured experimentally *in vivo*: concentration gradients of VEGF and its receptors. Most recently, a three-dimensional reaction-diffusion model of VEGF ligand–receptor kinetics and transport was developed to explore how VEGF isoform gradients form around angiogenic sprouts [29▪]. The model simulated what would happen to VEGF isoform gradients in tissue when the relative roles of heparan sulfate proteoglycans (HSPGs) vs. protease cleavage were varied. The study proposed an important reinterpretation of the relative roles of HSPGs vs. VEGF-cleaving proteases in the control of VEGF patterning *in vivo*: that the spatial localization of VEGF in tissues is governed by isoform-specific degradation or clearance instead of HSPG binding-mediated diffusion.

Our group recently developed a computational model of dynamic spatial transport of VEGF and its binding to receptors VEGFR-1 and VEGFR-2 in the context of a single blood vessel to investigate mechanisms of sprout emergence and guidance [31▪]. Specifically, we explored the hypothesis that endothelial secretion of soluble FMS-like tyrosine kinase-1 (sFlt-1) sequesters local VEGF and influences sprout emergence from parent vessels [6]. We ran simulations in which the lengths and distances between sprouts were intentionally and systematically varied, a feat that is difficult to accomplish *in vivo*. The model also computed important concentration gradients at resolutions that are not easily measured *in vivo*, such as active fetal liver kinase (Flk)-1 levels, free VEGF, and free sFlt-1. The model predicted that increased local sFlt-1 sequestration of VEGF results in decreased VEGF–Flk-1 levels but increases the relative gradient of VEGF–Flk-1 along the sprout surface, which could impact sprout spacing and emergence angle. Thus, although individual cell behaviors were not explicitly represented in this model, it predicted that sprouts influence how nearby sprouts perceive VEGF and affect 'guidance' in a sFlt-1-mediated manner.

A popular modeling approach that has been used to study cell behaviors and cell–cell communication in sprouting angiogenesis is agent-based modeling (ABM) [34,38]. Unlike the molecular kinetics models mentioned above, ABMs simulate discrete cells that behave autonomously according to a set of 'rules' that are derived from experimental findings. Frequently, these rules embody the stochastic, or random, behaviors of cells. The model output represents the emergent behavior of the system that results from the aggregate of interactions among individual cells. Bentley *et al.* [30] developed an agent-based model of VEGF tip cell induction that included inhibition by Notch/Delta-like 4 (Dll4). Importantly, the model predicted, for the first time, that lateral inhibition by Notch/Dll4 depends on the level of VEGFR-2 signaling. Moreover, in high VEGF concentrations, the model predicted a synchronous oscillation in tip/stalk phenotype across patches of cells; these oscillations are supported by fixed images of retinal vessels but remain to be proven.

Although many ABMs have been developed to aid in basic research [39,40▪,41▪], a recent ABM was designed to meet the more applied goal of designing vascularized tissue-engineered constructs. Artel *et al.* [33▪▪] developed an ABM that included sprout initiation, guidance, extension, and anastamosis to investigate the relationship between pore size of an ex-vivo scaffold and the rate of invasive angiogenesis. Scaffolds with larger pore size (160–270 mm) were predicted to accelerate angiogenesis, a finding that was validated against independent in-vitro experiments using porous poly(ethylene glycol) hydrogels.

Like ABMs, Cellular Potts (Glazier–Graner–Hogeweg) modeling simulates collections of discrete cells and has been applied to the study of angiogenesis. Most recently, this approach was employed to examine the role of contact-inhibited chemotaxis in sprout extension [32]. The model predicted that vascular endothelial cadherin-mediated contact inhibition of chemotaxis in the presence of a rapidly decaying secreted chemoattractant was sufficient to recapitulate aspects of capillary sprouting. It also underscored the importance of contact-dependent inhibition of pseudopod extension in generating proper branching and implicated vascular endothelial-cadherin in this process, which aligns nicely with experimental work in the murine allantois explant assay showing decreased endothelial cell motility with vascular endothelial-cadherin inhibition [42].

CONCLUSION

The increased sophistication of experimental tools and datasets has increased our knowledge of the players involved in angiogenic sprouting, and it has set the stage for utilizing these data, along with theoretical computational models, to better understand the interplay and integration of both signals and cell behaviors that characterize angiogenesis. In parallel, the increased robustness of computational models has already provided novel insights into regulation of blood vessel formation. The next era of analysis will focus on several challenges. First and foremost, we should use both experimental and computational tools to better understand the important signaling nodes at the molecular level, as well as the relevant cell behaviors that lead to productive sprouting; in this regard, considering angiogenesis as a multistaged process should be helpful. This will also require the development of new multiscale computational models that span genetic, molecular, and cell-to-tissue level interactions [35▪,43]. It is also important to understand how quantitative differences in parameters and graded responses affect spouting, as most current conceptual models are based on binary on/off inputs and outputs. Finally, it is a challenge to understand the temporal aspects of blood vessel sprouting; it is a dynamic process, but the time scale and parameters are poorly understood. In this challenge, both the high resolution live imaging now possible, and the ability to run simulations computationally, should be powerful tools to better elucidate the time dimension.

Acknowledgements

This work was supported by grants from the NIH (R01 HL43174 and HL86465 to V.L.B; R01 HL 082838 to S.M.P.; and R00 HL093219 to F.M.G.) and a grant from the Lineberger Comprehensive Cancer Center to V.L.B.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 236–237).

1. Risau W. Mechanisms of angiogenesis. Nature 1997; 386:671–674.
2. Gerhardt H, Golding M, Fruttiger M, *et al.* VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. J Cell Biol 2003; 161:1163–1177.
3. Hellstrom M, Phng LK, Hofmann JJ, *et al.* Dll4 signalling through Notch1 regulates formation of tip cells during angiogenesis. Nature 2007; 445:776–780.
4. Suchting S, Freitas C, le Noble F, *et al.* The Notch ligand Delta-like 4 negatively regulates endothelial tip cell formation and vessel branching. Proc Natl Acad Sci U S A 2007; 104:3225–3230.
5. Siekmann AF, Lawson ND. Notch signalling limits angiogenic cell behaviour in developing zebrafish arteries. Nature 2007; 445:781–784.
6. Chappell JC, Taylor SM, Ferrara N, Bautch VL. Local guidance of emerging vessel sprouts requires soluble Flt-1. Dev Cell 2009; 17:377–386.
7. Mazzone M, Dettori D, Leite de Oliveira R, *et al.* Heterozygous deficiency of PHD2 restores tumor oxygenation and inhibits metastasis via endothelial normalization. Cell 2009; 136:839–851.
8. ■ Armulik A, Genove G, Betsholtz C. Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. Dev Cell 2011; 21:193–215.

A recent comprehensive review of the pericyte field.

9. Wang S, Aurora AB, Johnson BA, *et al.* The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. Dev Cell 2008; 15:261–271.
10. ■ Zhou Q, Gallagher R, Ufret-Vincenty R, *et al.* Regulation of angiogenesis and choroidal neovascularization by members of microRNA-23∼27∼24 clusters. Proc Natl Acad Sci U S A 2011; 108:8287–8292.

This study reveals a requirement for miRNA 23/27 in mammalian sprouting angiogenesis via negative modulation of antiangiogenic Sprouty2 and Sema6A.

11. ■■ Nicoli S, Standley C, Walker P, *et al.* MicroRNA-mediated integration of haemodynamics and Vegf signalling during angiogenesis. Nature 2010; 464:1196–1200.

This study shows that blood flow is required for aortic arch development in zebrafish, and that mir-126 integrates the physiological stimulus with growth factor signaling.

12. ■ Guarani V, Deflorian G, Franco CA, *et al.* Acetylation-dependent regulation of endothelial Notch signalling by the SIRT1 deacetylase. Nature 2011; 473:234–238.

This study shows that the Notch intracellular domain is a substrate of sirtuin 1 (SIRT1), which destabilizes Notch intracellular domain and attenuates Notch signaling; loss of SIRT1 attenuates vessel branching through elevated Notch.

13. Iruela-Arispe ML, Davis GE. Cellular and molecular mechanisms of vascular lumen formation. Dev Cell 2009; 16:222–231.
14. Blum Y, Belting HG, Ellertsdottir E, *et al.* Complex cell rearrangements during intersegmental vessel sprouting and vessel fusion in the zebrafish embryo. Dev Biol 2008; 316:312–322.
15. Strilic B, Kucera T, Eglinger J, *et al.* The molecular basis of vascular lumen formation in the developing mouse aorta. Dev Cell 2009; 17:505–515.
16. ■ Xu K, Sacharidou A, Fu S, *et al.* Blood vessel tubulogenesis requires Rasip1 regulation of GTPase signaling. Dev Cell 2011; 20:526–539.

This article shows that Rho GTPase signaling is important for vessel lumen formation, and Rasip1 negatively modulates Rho signaling; loss of Rasip1 prevents proper apical-basal polarization and tube formation.

17. ■ Fantin A, Vieira JM, Gestri G, *et al.* Tissue macrophages act as cellular chaperones for vascular anastomosis downstream of VEGF-mediated endothelial tip cell induction. Blood 2010; 116:829–840.

This study shows that tissue macrophages can promote the fusion of two sprouts to form a new connection, and that the 'molecular signature' of these macrophages resembles that of proangiogenic tumor macrophages.

18. ■■ Bauer AL, Jackson TL, Jiang Y, Rohlf T. Receptor cross-talk in angiogenesis: mapping environmental cues to cell phenotype using a stochastic, Boolean signaling network model. J Theor Biol 2010; 264:838–846.

This article applies Boolean inputs to a rigorously defined model of intracellular signaling cascades to predict cell phenotype under conditions of tumor angiogenesis. The network predicts phenotypes such as apoptotic, proliferative, migratory, etc., by integrating inputs from cadherins, integrins, and growth factor receptors. Within the computational model the signaling network is systematically probed for potential drug targets using hypothetical (i.e., not yet developed) inhibitors.

19. ■■ Swanson KR, Rockne RC, Claridge J, *et al.* Quantifying the role of angiogenesis in malignant progression of gliomas: in silico modeling integrates imaging and histology. Cancer Res 2011; 71:7366–7375.

This article combines computational modeling with molecular biology data across multiple length scales to predict malignant progression of glioblastomas with validation against clinical data from three individual patients. Of note, the model predicts that accumulation of genetic mutations is not required for progression to advanced stages of disease; rather, a range of fixed proliferation and invasion rates determine progression based on initial lesionsize and degree of hypoxia and necrosis.

20. ■ Jackson T, Zheng X. A cell-based model of endothelial cell migration, proliferation and maturation during corneal angiogenesis. Bull Math Biol 2010; 72:830–868.

This article uses both mechanical and molecular inputs to build a model of cell proliferation and migration in corneal angiogenesis. The computational model reveals theoretical limits of vessel growth when proliferation is inhibited.

21. ■■ Das A, Lauffenburger D, Asada H, Kamm RD. A hybrid continuum-discrete modelling approach to predict and control angiogenesis: analysis of combinatorial growth factor and matrix effects on vessel-sprouting morphology. Philos Trans R Soc A Math Phys Eng Sci 2010; 368:2937–2960.

This article discusses the construction of a robust multiscale model of sprouting angiogenesis based on cell-state transitions, intercellular communication, and stochastic single-cell processes. The model-building procedure is thoroughly described and validated against highly controlled in-vitro microfluidics experiments. Through reduction of inputs in both the in-vitro and in-silico models, the article posits general categories of sprout morphology and mechanisms for controlling these morphologies.

22. Fruttiger M. Development of the retinal vasculature. Angiogenesis 2007; 10:77–88.
23. Kamei M, Isogai S, Pan W, Weinstein BM. Imaging blood vessels in the zebrafish. Methods Cell Biol 2010; 100:27–54.
24. Nakatsu MN, Davis J, Hughes CC. Optimized fibrin gel bead assay for the study of angiogenesis. J Vis Exp 2007; (3):186.
25. Kearney JB, Bautch VL. In vitro differentiation of mouse ES cells: hematopoietic and vascular development. Methods Enzymol 2003; 365:83–98.
26. Jakobsson L, Kreuger J, Claesson-Welsh L. Building blood vessels: stem cell models in vascular biology. J Cell Biol 2007; 177:751–755.
27. Yin Z, Noren D, Wang CJ, *et al.* Analysis of pairwise cell interactions using an integrated dielectrophoretic-microfluidic system. Mol Syst Biol 2008; 4:232.
28. Chang CC, Hoying JB. Directed three-dimensional growth of microvascular cells and isolated microvessel fragments. Cell Transplant 2006; 15:533–540.
29. ■ Vempati P, Popel AS, Mac Gabhann F. Formation of VEGF isoform-specific spatial distributions governing angiogenesis: computational analysis. BMC Syst Biol 2011; 5:59.

This article leverages computational models of extracellular matrix chemokine gradients to interpret in-vivo data and propose mechanistic explanations for isoform-specific degradation of VEGF. The three-dimensional model predicts differences in isoform responsiveness are effective at different length scales for vascular patterning.

30. Bentley K, Gerhardt H, Bates PA. Agent-based simulation of notch-mediated tip cell selection in angiogenic sprout initialisation. J Theor Biol 2008; 250:25–36.
31. ■ Hashambhoy YL, Chappell JC, Peirce-Cottler SM, *et al.* Computational modeling of interacting VEGF and soluble VEGF receptor concentration gradients. Front Physiol 2011; 2:62.

This article uses computational modeling to evaluate VEGF and soluble VEGF receptor gradients at high resolution. In-silico techniques permit investigation of single tip cell or multitip cell geometries and their effects on chemokine diffusion.

32. Merks RM, Perryn ED, Shirinifard A, Glazier JA. Contact-inhibited chemotaxis in de novo and sprouting blood-vessel growth. PLoS Comput Biol 2008; 4:e1000163.
33. ■■ Artel A, Mehdizadeh H, Chiu YC, *et al.* An agent-based model for the investigation of neovascularization within porous scaffolds. Tissue Eng Part A 2011; 17:2133–2141.

This article notably applies agent based modeling to the field of tissue engineering by evaluating the contribution of pore size to angiogenesis in polymer scaffolds. A thorough description of the modeling approach is provided, as well as insight into rule generation from literature sources and in-vivo experimentation. Using training data from in-vivo studies in the absence of polymer, the model predicts vessel growth kinetics across multiple scaffold porosities and is validated against independent test data sets.

34. Peirce SM, Van Gieson EJ, Skalak TC. Multicellular simulation predicts microvascular patterning and in silico tissue assembly. FASEB J 2004; 18:731–733.

35. Liu G, Qutub AA, Vempati P, *et al.* Module-based multiscale simulation of ■ angiogenesis in skeletal muscle. Theor Biol Med Model 2011; 8:6.
This article combines multiple modeling techniques (termed 'modules') to investigate angiogenesis at multiple spatial scales. It exemplifies the strengths of model integration across broad scale domains.
36. Stefanini MO, Qutub AA, Mac Gabhann F, Popel AS. Computational models ■ of VEGF-associated angiogenic processes in cancer. Math Med Biol 2011. doi:10.1093/imammb/dqq025. [Epub ahead of print]
This review provides insight into the multiple scales of computational modeling currently employed in the field of tumor angiogenesis. The article highlights key findings in systems biology and their most recent application.
37. Wu FT, Stefanini MO, Mac Gabhann F, *et al.* VEGF and soluble VEGF ■ receptor-1 (sFlt-1) distributions in peripheral arterial disease: an in silico model. Am J Physiol Heart Circ Physiol 2010; 298:H2174–H2191.
This article uses a three-compartment model of peripheral arterial disease to investigate macromolecular transport of VEGF and soluble VEGF receptor-1. It is notable for gleaning insight into a microscopic disease process using computational models on a whole-organism scale.
38. Qutub AA, Popel AS. Elongation, proliferation & migration differentiate endothelial cell phenotypes and determine capillary sprouting. BMC Syst Biol 2009; 3:13.
39. Bailey AM, Lawrence MB, Shang H, *et al.* Agent-based model of therapeutic adipose-derived stromal cell trafficking during ischemia predicts ability to roll on P-selectin. PLoS Comput Biol 2009; 5:e1000294.
40. Thorne BC, Hayenga HN, Humphrey JD, Peirce SM. Toward a multiscale ■ computational model of arterial adaptation in hypertension: verification of a multicell agent based model. Front Physiol 2011; 2:20.
This article proposes a novel rule-scoring system for agent based modeling as applied to a model of the artery wall under homeostatic and hypertensive conditions. Provides an excellent overview of model construction and implementation.
41. Deisboeck TS, Wang Z, Macklin P, Cristini V. Multiscale cancer modeling. ■ Annu Rev Biomed Eng 2011; 13:127–155.
This review article provides excellent examples of computational modeling across multiple temporal and spatial scales in the field of cancer biology. It highlights cutting-edge modeling techniques and emphasizes the power of multiscale modeling in the context of tumor growth.
42. Perryn ED, Czirok A, Little CD. Vascular sprout formation entails tissue deformations and VE-cadherin-dependent cell-autonomous motility. Dev Biol 2008; 313:545–555.
43. Qutub AA, Mac Gabhann F, Karagiannis ED, *et al.* Multiscale models of angiogenesis. IEEE Eng Med Biol Mag 2009; 28:14–31.