Published in final edited form as:
Curr Opin Hematol. 2012 May ; 19(3): 184–191. doi:10.1097/MOH.0b013e3283523ea6.

Integration of Experimental and Computational Approaches to Sprouting Angiogenesis

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Abstract

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 VEGF 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

Sprouting angiogenesis; experimental models; computational models; stages of angiogenesis

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Conflict of Interest: None.

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) 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 while 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 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 (ES) 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 (Figure 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.

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 pro-angiogenic 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, while 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 micro RNAs 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 [14-16*]. 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 multi-staged process (Figure 2) has been facilitated by *in vitro* and *in vivo* assays that are 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 post-natal 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 post-natal week [18]. 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 [19]. 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 HUVEC-coated beads in a fibrin matrix sprout and form lumenized vessels [20]. Another model involves a programmed differentiation of mouse ES cells to form lumenized vessels over the course of a week [21]. In a variant of this model partially differentiated ES cell-derived embryoid bodies are embedded in collagen with added VEGF-A, and developing vessels sprout outward into the matrix [22].

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 set-ups (i.e. micro-fluidics chambers) have provided quantitative and spatial information about signaling cues [23, 24]. It is apparent, however, that a full understanding of how angiogenic sprouting and vessel network formation is 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 (Figure 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: 1) build the computational model based on experimentally-derived knowledge (e.g. obtain model parameters directly from empirical data), 2) validate the model by comparing its predictions to independent experimental parameters (i.e. not ones that were used to construct the model), 3) 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 (Figure 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 about VEGF signaling within tumors [25*] and muscle [26*], primarily because of their ability to predict what cannot be measured experimentally *in vivo*: concentration gradients of VEGF and its receptors. Most recently, a 3-dimensional reaction-diffusion model of VEGF ligand-receptor kinetics and transport was developed to explore how VEGF isoform gradients form around angiogenic sprouts [27*]. 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 [28*]. Specifically, we explored the hypothesis that endothelial secretion of sFlt-1 sequesters local VEGF and influences sprout emergence from parent vessels [6]. We ran simulations where 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 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, while 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) [29, 30]. 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 amongst individual cells. Bentley et al. developed an agent-based model of

VEGF tip cell induction that included inhibition by Notch/Dll4 [31]. Importantly, the model predicted, for the first time, that lateral inhibition by Notch/Dll4 is 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.

While many ABMs have been developed to aid in basic research [32, 33*, 34*], a recent ABM was designed to meet the more applied goal of designing vascularized tissue engineered constructs. Cinar et al. 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 [35**]. 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 [36]. The model predicted that VE-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 VE-cadherin in this process, which aligns nicely with experimental work in the murine allantois explant assay showing decreased endothelial cell motility with VE-cadherin inhibition [37].

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 multi-staged process should be helpful. This will also require the development of new multi-scale computational models that span genetic, molecular, and cell-to-tissue level interactions [38, 39*]. It is also important to understand how quantitative differences in parameters and graded responses affect spouting, since 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.

Acknowledgments

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. The authors would like to acknowledge Joseph Walpole for his assistance with preparing the recommended reading annotations.

Funding: 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.

Abbreviations

VEGF	vascular endothelial growth factor
ABM	Agent-based Model
HSPG	heparan sulfate proteoglycan

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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

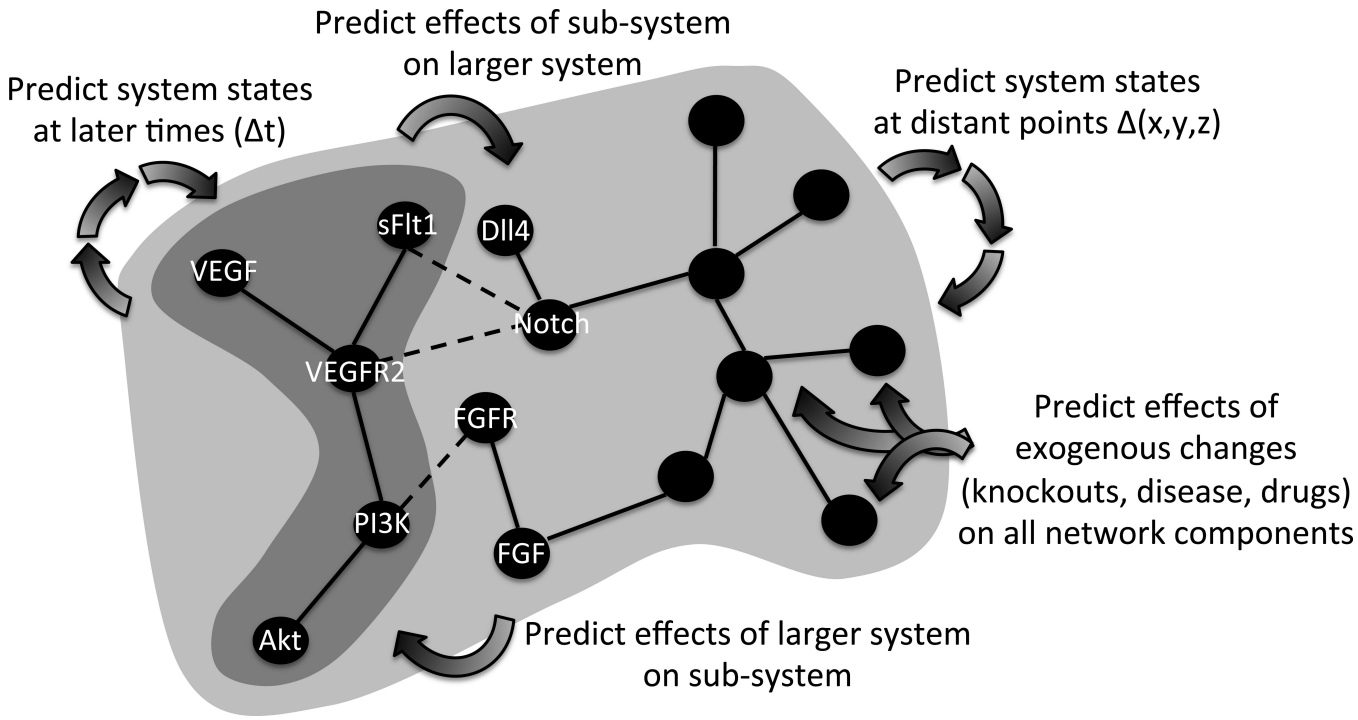

Fig. 1. A systems-level perspective to studying angiogenesis

Legend: The typical reductionist approach enables close examination of a few interacting signals (dark gray) within a sub-module 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).

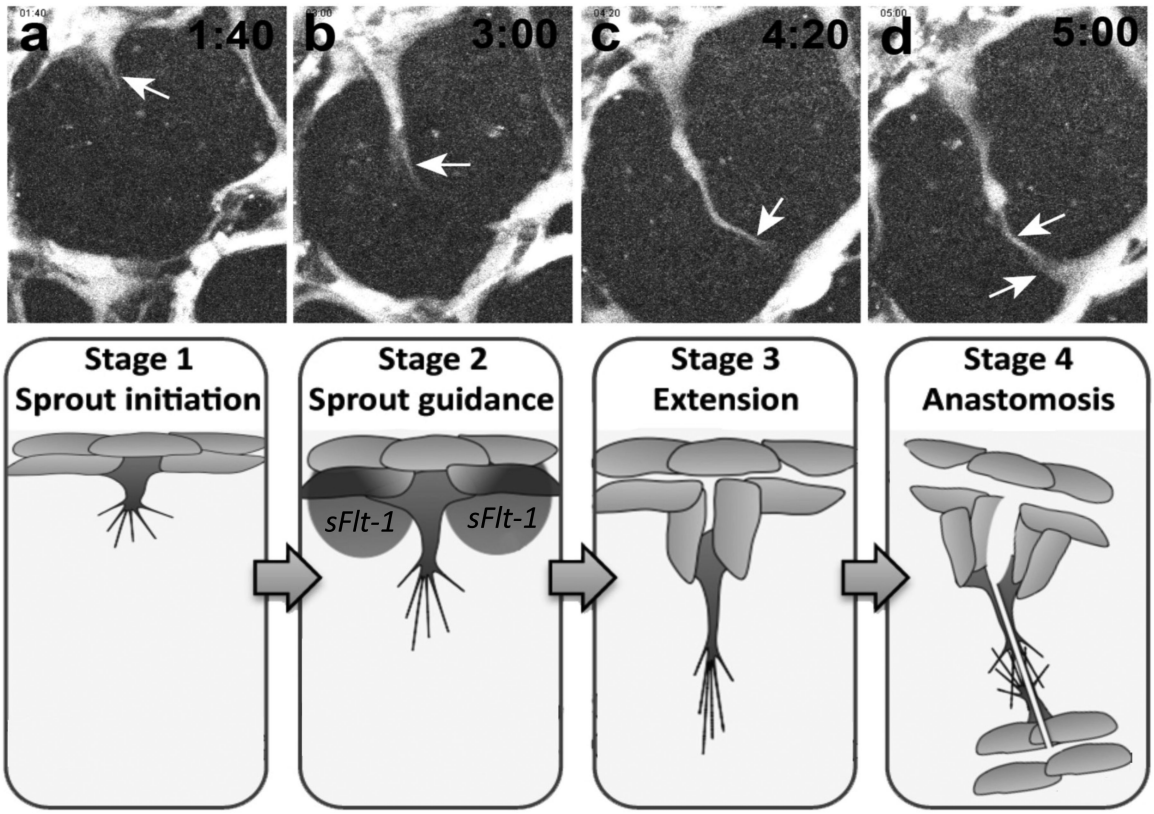

Fig. 2. Stages of angiogenesis

Legend: Time lapse images of ES 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 ES cell-derived vessels have been genetically modified to express eGFP and visualized using confocal microscopy. Time stamps = time elapsed (hr) since start of run. (Images courtesy of Dr. John Chappell, Ph.D., University of North Carolina)

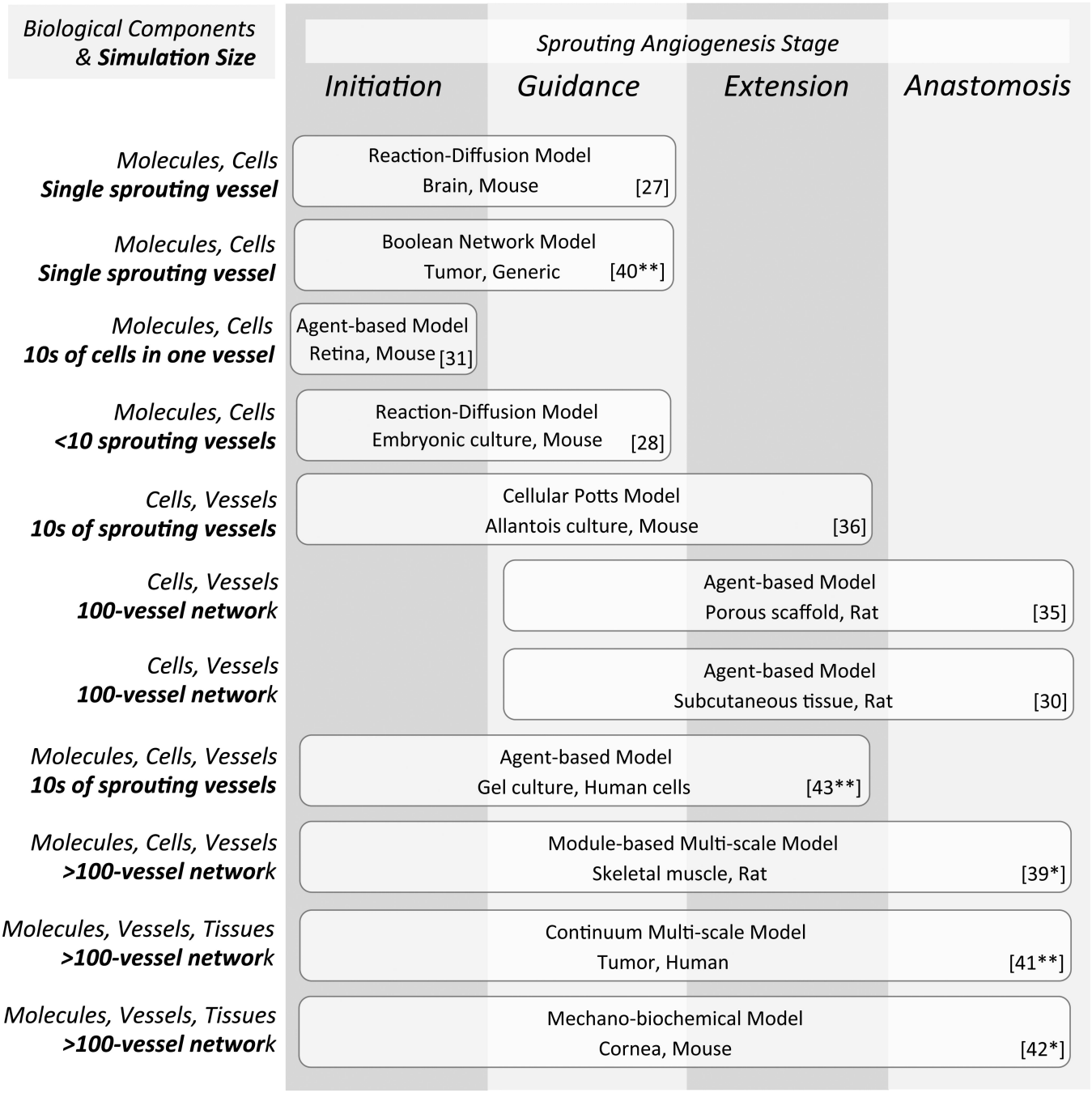

Fig. 3. Summary of computational models of angiogenesis that span different stages

Legend: Each of the multi-scale 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 multi-vessel network. In each case the citation number refers to the reference list in the main text.