

# Multiscale Computational Modeling in Vascular Biology: From Molecular Mechanisms to Tissue-Level Structure and Function

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**Abstract** Blood vessels exhibit a remarkable ability to adapt in response to sustained alterations in hemodynamic loads and diverse disease processes. Although such adaptations typically manifest at the tissue level, underlying mechanisms exist at cellular and molecular levels. Dramatic technological advances in recent years, including sophisticated theoretical and computational modeling, have enabled significantly increased understanding at tissue, cellular, and molecular levels, yet there has been little attempt to integrate the associated models across these length and time scales. In this chapter, we suggest a new paradigm for identifying strengths and weaknesses of models at different scales and for establishing congruent models that more completely predict vascular adaptations. Specifically, we show the importance of linking intracellular with cellular models and cellular models with tissue level models. In this way, we propose a new approach for

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incorporating events across these three levels, thus providing a means to predict phenomena that can only emerge from a system of integrated interactions.

## 1 Introduction: Vascular Biology as a Complex System

Vascular development, adaptations to altered hemodynamics, the progression of disease, and responses to injury or clinical treatment—in each of these cases, one can identify tissue-level changes in geometry, structure, function, and properties that result from altered cellular phenotypes, which in turn depend on changes in intracellular signaling pathways. Indeed, our knowledge of the complex web of signals that underpin vascular function, homeostasis, growth, and remodeling at different levels of biological scale is growing exponentially as more sophisticated experimental models, techniques for analysis, and tools for integrating data become available. Recent technological developments in molecular biology and bioinformatics have thus made high-throughput analyses commonplace and “-omics” data widely accessible. The empirical tools we can use to manipulate and measure vascular structure, function, and adaptation *in vivo*—ranging from inducible genetic manipulations in mice to non-invasive intravital microscopy with single-cell resolution—are more flexible and precise than ever before. Parallel advances in systems biology, agent based modeling, continuum biomechanics, and computational methods have enabled significantly increased understanding of vascular biology at molecular, cellular, and tissue levels.

Despite all of these advances, critical questions in vascular mechanobiology—that is, many of the big questions that impact the care of thousands of patients each year—remain unanswered. For example, how do medial vascular smooth muscle cells (SMC) transduce mechanical stimuli in a way that impacts their production and secretion of proteases that degrade the extracellular matrix (ECM)? How, in turn, does degradation of ECM liberate growth factors that impact the proliferation of adventitial fibroblasts? What mechanical stimuli induce endothelial-to-mesenchymal transition and how does this perturb homeostasis? Similarly, many questions remain regarding interactions between wall mechanics and pharmacological treatments. What impact, for example, would a calcium channel blocker have on the stiffness of an arterial wall in the presence of a stiff atherosclerotic plaque that occludes 50 % of the lumen? While it is no small task to study such questions in isolation, the prospect of conceptualizing how these phenomena interact in space and time to create an emergent response is even more daunting. Indeed, the difficulty in answering these questions arises not from our ignorance of the individual components that are relevant in this complex system, but rather from the way in which they integrate to produce emergent outcomes. Even when we understand singular cause-and-effect relationships between two components, we face the challenge of integrating sets of relationships across different spatiotemporal scales in these complex systems. We submit that achieving a more holistic understanding

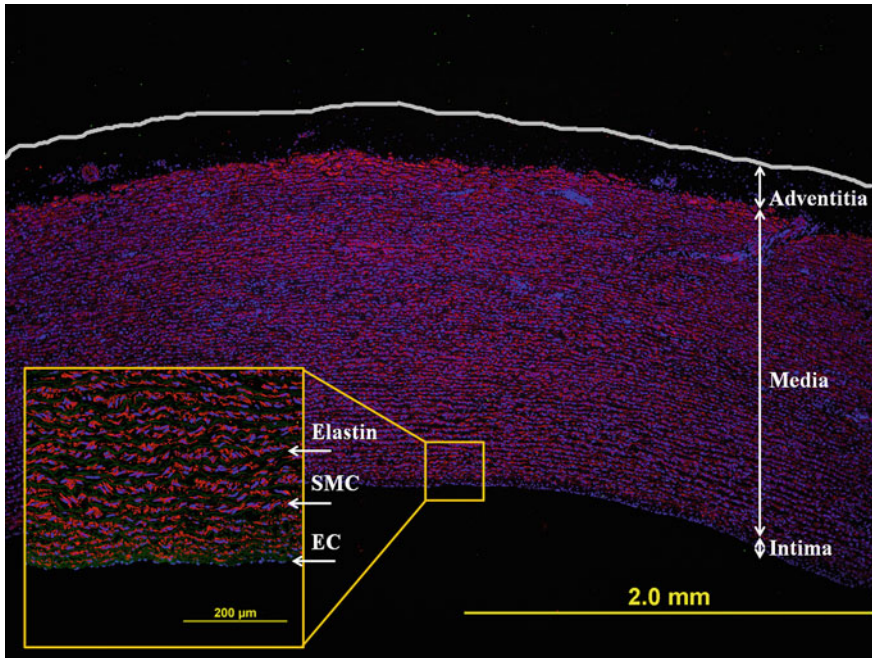
of how blood vessels develop, maintain homeostasis, and respond to diseases requires an approach that couples experiments with theoretical and computational models and integrates processes across multiple length and time scales in a way that ultimately captures the emergent behaviors of the complex system that we know as a blood vessel.

This chapter will first provide an overview of vascular wall structure and many of the well characterized molecular signals that underpin functional and dysfunctional cellular behaviors in vascular tissues. We will then briefly describe some theoretical approaches, including continuum biomechanics, agent-based modeling, and models of intracellular signaling, that have been applied at different spatial scales to the study vascular function and adaptation. Examples from the literature will be highlighted to depict how modeling has been fruitful in producing new understanding at each level of spatial scale. We will then present our fundamental premise: that integration of processes across scales, as enabled by truly integrated, multiscale computational models, can result in a new understanding of emergent behaviors in vascular biology. This multiscale modeling approach, in turn, is expected to reveal new categories of questions that can be posed—questions that embrace multi-dimensional cause-and-effect relationships. We have developed the conceptual basis for such a multiscale model and begun integration efforts for a combined tissue-level continuum mixture and multi-cell agent-based model as well as for a combined agent-based and intracellular signaling model. We will conclude with a summary of our goal to integrate models from continuum to intracellular scales and to highlight some of the opportunities and challenges posed by integrative multiscale modeling of complex systems.

## 2 Background

### 2.1 Vascular Wall Structure

The microstructure of arteries and veins varies with species, age, disease, and location along the vascular tree [30], yet the normal wall in maturity is characterized by three primary layers—the intima, media, and adventitia (Fig. 1). The intima, or inner layer, consists of a monolayer of endothelial cells (EC) and an underlying basal lamina composed of mesh-like type IV collagen and adhesion molecules such as laminin. In addition to being a smooth, nonthrombogenic interface between the blood and contents of the wall, the endothelium is biologically active. In response to chemical and mechanical stimuli, ECs produce a host of vasoactive molecules (which control vascular dilatation or constriction), growth factors (which promote cell replication or synthesis of proteins), proteases (which degrade proteins), and factors that regulate local immune responses and clotting processes. The endothelium also modulates transport of substances into the wall (e.g., white blood cells or lipids), and thereby plays important roles in diseases



**Fig. 1** Porcine aorta displaying the three arterial layers: thin intima, media, and thin adventitia. The monolayer of endothelial cells is revealed by the *blue lining* (cell nuclei) in the large image. The smooth muscle cells (SMC) are shown in *red* (alpha smooth muscle actin staining) and the layered elastin in *green*, which separates each SMC layer. One vessel of the vasa vasorum is seen in the *upper left* of the large image as a round collection of SMCs

such as atherosclerosis. Realization that many functions of the endothelium correlate with changes in hemodynamic loads provided important guidance for treating many vascular disorders and renewed interest in the biomechanics even though this layer contributes little to the overall structural integrity of the wall. Flow induced wall shear stresses tend to be on the order of 1.5 Pa in arteries and 0.15 Pa in veins; the mean value of this stress can be estimated *in vivo* from measurements of viscosity, volumetric flow-rate, and luminal radius.

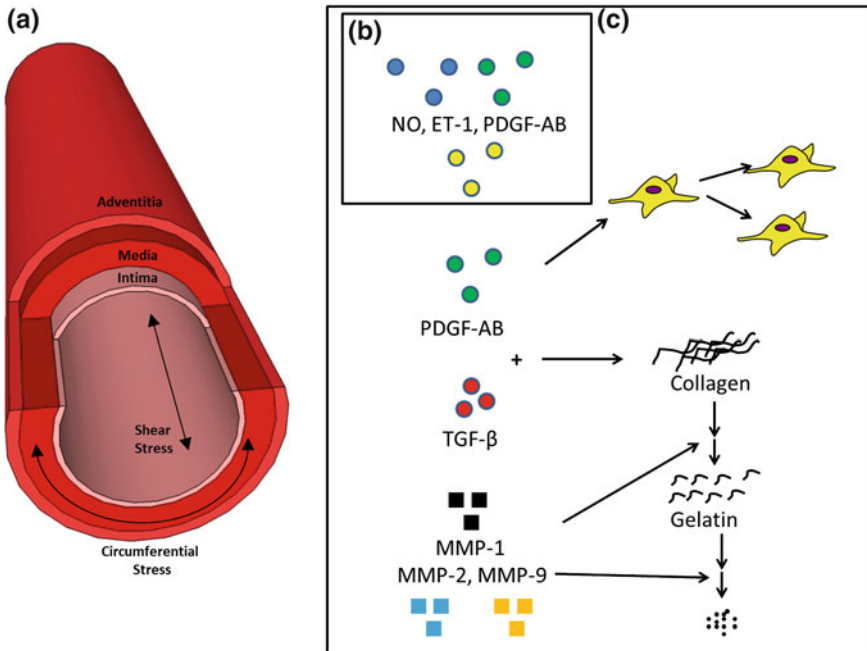
The media, or middle layer, consists primarily of SMCs embedded in ECM consisting of elastic fibers, various types of collagen (I, III, V, etc.), and proteoglycans (Fig. 1). In general, the closer these vessels are to the heart the more elastin (the main constituent of elastic fibers), and the farther away the more smooth muscle. Regardless, the mean circumferential wall stress tends to be on the order of 100 kPa, which can be estimated *in vivo* via measurements of transmural pressure, inner radius, and wall thickness. Whereas SMCs primarily synthesize proteins of the ECM during development and disease, they endow the normal mature wall with an ability to constrict or dilate and thereby regulate blood flow locally. Smooth muscle contraction, hypertrophy (increase in size), hyperplasia

(increase in number), apoptosis (cell suicide), and migration (often from the media to subintima) play essential roles in diseases such as aneurysms, atherosclerosis, and hypertension. Loss of matrix proteins, particularly elastin, similarly plays key roles in the formation of aneurysms or dissections.

The adventitia, or outer layer, often merges with the perivascular tissue. It consists primarily of fibroblasts and axially oriented type I collagen, but may include admixed elastic fibers, nerves, and its own small vasculature, the vasa vasorum, when the thickness of the wall is too great to allow sufficient transmural diffusion of oxygen directly from the blood. Fibroblasts are responsible primarily for regulating the matrix, particularly collagen, but they can migrate, proliferate, and differentiate. Indeed, there is growing evidence that migrating fibroblasts play significant roles in many diseases. Nevertheless, the normal adventitia is thought to serve, in large part, as a protective sheath that prevents over-distension of the media; like all muscle, smooth muscle contracts maximally at a certain length, above and below which the contractions are less forceful. Finally, the adventitia is typically demarcated from the media by an external elastic lamina (except in cerebral arteries); the media is similarly demarcated from the intima by an internal elastic lamina, which is a fenestrated, cylindrical sheet of elastin.

Cross-linked elastin is one of the most stable proteins in the body; it endows vessels with considerable elasticity over finite deformations (e.g., nearly linear stress response over stretches of 150–200 %) and it helps control the phenotype of the SMCs. Specifically, cross-linked elastin encourages a quiescent, contractile phenotype characteristic of maturity. This is in contrast to effects of the elastin precursor, tropoelastin, which is not cross-linked, contributes little to the structural integrity, and encourages smooth muscle migration, proliferation, and synthesis of extracellular matrix, particularly in development. The collagens are the primary family of structural proteins in the body, with fibrillar types I and III endowing tissues with significant tensile stiffness. Collagen fibers turn over continuously and thereby play key roles in homeostasis and remodeling. They can be on the order of microns in diameter and are often undulated slightly in the normal physiologic state; they manifest their high stiffness when straightened. Proteoglycans represent a large class of molecules having diverse functions. Structurally, they tend to be most important in sequestering water within the tissue, which, as in cells, typically accounts for  $\sim 70$  % of the total mass. To provide a better idea of relative distributions of these various constituents, the media of the thoracic aorta (cf. Fig. 1) consists of, by dry weight,  $\sim 37$  % collagen, 33 % SMCs, 24 % elastin, and 6 % other constituents whereas the adventitia consists of  $\sim 78$  % collagen, 9 % fibroblasts, 2 % elastin, and 11 % other constituents. Nevertheless, each vessel has different distributions of constituents within each layer and different relative thicknesses of the media and adventitia; overall structural integrity is thus differentially controlled by balances and imbalances in cell and matrix turnover (Fig. 2). For more on vascular ECM and its relation to mechanics, see [62].

Vascular structure, function, and material properties are dictated by the three primary cell types of the wall (endothelial, smooth muscle, and fibroblasts) and in some cases cells from the blood stream (e.g., platelets, monocytes, progenitor



**Fig. 2** Illustrative arterial mechanics and molecular interactions. **a** The three layers of an artery wall. The Intima is composed of endothelial cells and a basement membrane; the Media, smooth muscle cells and primarily elastin and collagen; the Adventitia, fibroblasts and extracellular matrix. Shear stress typically orients in the axial direction, while intramural stresses are circumferential and axial in orientation. **b** Factors governed by shear stress and produced by endothelial cells include NO, ET-1, and PDGF-AB. **c** Factors governed by circumferential stress and produced by smooth muscle cells include PDGF-AB, TGF- $\beta$ , and MMPs. Illustrative effects of these growth factors and MMPs are shown to the *right*. PDGF-AB and TGF- $\beta$  act through complex intracellular pathways to promote collagen production and cell proliferation or phenotypic switching, while MMPs degrade collagen, gelatin, and elastin

cells). That ECs respond directly to changing mechanical loads was first noted  $\sim 35$  years ago and is now a well-documented example of mechanotransduction—that is, the sensing and converting of mechanical stimuli into a signal that controls gene expression and hence cellular activities. For example, ECs increase their production of vasodilators (e.g., nitric oxide (NO) and prostacyclin (PGI<sub>2</sub>)) in response to increases in wall shear stress; this allows the vessel to dilate, thereby decreasing the resistance to flow, and promotes endothelial proliferation to cover the increased surface area of the dilated vessel. Conversely, ECs increase production of vasoconstrictors (e.g., endothelin-1 (ET-1) and angiotensin-II (ANG-II)) in response to decreases in flow or increases in pressure. Endothelial cells also produce a host of growth regulatory molecules (including vascular endothelial growth factor (VEGF), platelet derived growth factors (PDGF), fibroblast growth factors (FGF)), adhesion molecules (including vascular cell adhesion molecule (VCAM-1) and intercellular adhesion molecule (ICAM-1)),

cytokines and chemokines (e.g., interleukin-1, IL-1, and monocyte chemoattractant protein, MCP-1), and clotting factors (e.g., tPA)—all in response to changing mechanical stimuli including local stresses or trauma. See Refs. [19, 28] for more on endothelial mechanobiology.

The structure of smooth muscle differs from that of skeletal and cardiac muscle, but its contractility also depends on a calcium dependent actin-myosin interaction. Vascular smooth muscle can generate contractile forces comparable to those of striated muscles while maintaining the contraction for longer periods and at a lower expenditure of energy. This feature allows blood vessels to maintain a “basal tone” from which they can dilate or constrict further. Like ECs, SMCs respond to changes in their mechanical environment; for example, SMCs alter their synthesis of collagen in response to changes in mechanical loading. Mechanical damage to elastin can also induce phenotypic changes in smooth muscle that promote migration, proliferation, and apoptosis in addition to synthesis of matrix. This causality appears to be fundamental to the response of the arterial wall to clinical interventions such as balloon angioplasty and stenting and likewise to the response of the venous wall to its clinical use as an arterial by-pass graft. See Refs. [42, 68] for more on the mechanobiology of smooth muscle.

Fibroblasts are primarily responsible for regulating the extracellular matrix in the adventitia, as, for example, via synthesis and degradation of collagen. Degradation is accomplished via ingestion by cells (phagocytosis) or the release of enzymes, including the matrix metalloproteinases (MMPs). Fibroblasts play an important role in regulating the ECM in many soft tissues (from the eye to the skin to heart tissue) and are easily studied *in vitro*. For these reasons, there is a considerable literature on the mechanobiology of fibroblasts and myofibroblasts (e.g., [59]). Macrophages are scavenger cells; in response to a local injury, they enter the vessel wall from the blood (actually blood borne monocytes adhere to the wall and transform into macrophages while inside the wall) and act primarily via phagocytosis or the release of MMPs. They, too, are responsive to changes in mechanical stimuli [64]. Platelets also circulate within the bloodstream; they play a key role in coagulation, but also release growth factors (e.g., PDGF) and vasoconstrictors (e.g., serotonin, 5-HT, and thromboxane, TXA<sub>2</sub>) that affect both ECs and SMCs. Platelet derived vasoconstrictors play a particularly damaging role following the rupture of intracranial aneurysms, causing nearby vessels to constrict and cause distal strokes. More specifics of the molecular biology of blood borne cells can be found in general textbooks.

## ***2.2 Key Signaling Pathways in Vascular Adaptation***

Myriad signaling pathways play important roles in vascular homeostasis and adaptation in both large and small vessels. Many of these molecules are homologous across species and play important roles in mediating homeostasis and growth in other organ systems. For example, VEGF is a highly conserved family

**Table 1** Some of the key molecules produced and/or expressed by vascular cells in response to altered hemodynamic loading, disease, and injury

<b>Vasoactive molecules</b>	<b>Cytokines</b>
Nitric oxide	IL-1
Endothelin-1	IL-6
Angiotensin-II	IL-8
Serotonin	IL-10
Thromboxane	SDF-1
Thrombin	
<b>Growth factors/receptors</b>	<b>Proteinases and modulators</b>
PDGF-BB/PDGFR-alpha, beta	MMP-2
VEGF/Fik-1, Flt-1, neuropilin	MMP-9
TGF- $\beta$ 1/TGFBRI/II	MMP-1
bFGF/FGF-R	TIMP-1
EphrinB2/EphB4	TIMP-2
<b>Cell-cell adhesion</b>	<b>ECM proteins</b>
P-selectin/PSGL-1	Type I collagen
E-selectin/PSGL-1	Type III collagen
CD-34/L-selectin	Type IV collagen
VCAM-1/VLA-4	Elastin/Microfibrils
ICAM-1/LFA-1 & MAC-1	Laminin/Fibronectin

of molecules present in zebrafish, mice, and humans, and is a pivotal regulator of growth and patterning in both the circulatory and nervous systems.

The key signaling pathways in the vascular tree can be broadly lumped into functional categories based on their ability to mediate vascular tone (vasoactive molecules), activate cells (cytokines), induce growth (growth factors), form and impact the extracellular milieu (ECM molecules and proteinases), and orchestrate cell adhesion (Table 1). While a comprehensive review of all of these key signaling pathways is beyond the scope of this chapter, we highlight some of the most widely studied molecules that play a diverse set of context-specific roles in the vasculature. We thus focus on a small sub-set of signaling pathways that are particularly important in mediating vascular responses to physiological and pathological alterations and those that are most relevant in our proposed multiscale model of hypertension, which is discussed in subsequent sections.

NO is one of the most widely studied signaling molecules in the vasculature. It exerts its effects at both systemic and cellular levels throughout the microcirculation and in larger vessels throughout the body. This highly diffusible and short-lived free radical gas is synthesized via nitric oxide synthases [35], a family of enzymes that convert L-arginine to NO (for review of this process in ECs see [54]). Impaired NO activity, due to decreased synthesis or increased degradation, is a hallmark of endothelial dysfunction and has been observed in a host of conditions and diseases ranging from aging to atherosclerosis, hypertension, and diabetes [47]. NO is a potent vasodilator [12] and thus regulates vascular SMC tone. Both exogenous NO [48, 72] and over-expression of endothelial nitric oxide synthase



(eNOS) promote angiogenesis [56]. Interestingly, NO and eNOS also upregulate and are synergistic with pro-angiogenic growth factors, such as VEGF and Angiopoietin-1 [10]. This relationship supports an intriguing mechanistic linkage between hemodynamic alterations due to vasodilation and growth factor mediated angiogenesis. NO also plays a role in mediating microvascular permeability (for review see [22]) and inflammation by inhibiting platelet adhesion, aggregation, and leukocyte adhesion [24].

NO production via eNOS has long been associated with the mechanical shear stress experienced by the vascular endothelium of large vessels (e.g., during sustained changes in blood flow) and is critical for blood flow-dependent adaptive remodeling of the media [50], yet a direct molecular linkage between NO and SMC remodeling (proliferation and apoptosis) was only recently discovered by Yu et al. [69]. They demonstrated that abnormal flow-dependent remodeling in eNOS knockout mice is associated with activation of the PDGF signaling pathway and downstream inhibition of apoptosis. Moreover, they showed that NO negatively regulates PDGF-induced cell proliferation in vascular SMCs. Hence, this signaling module represents yet another example of the mechanistic linkages between mechanical forces experienced by blood vessels, diffusible signals and morphogens secreted by cells, and defined cellular behaviors that have important consequences on long-term vascular tissue structure and function.

As mentioned above, PDGF is a family of growth factors synthesized and secreted by vascular ECs and SMCs in homodimeric (e.g., PDGF-AA and PDGF-BB) and heterodimeric (e.g., PDGF-AB) forms [20]. PDGF homodimers and heterodimers bind to dimeric tyrosine kinase receptors, PDGFR-alpha and PDGFR-beta, with different affinities. PDGF is a potent mitogen for SMCs and fibroblasts, stimulating proliferation, migration, and preventing apoptosis [41] (Fig. 2). Dysfunction of the PDGF signaling pathway has been implicated in a number of diseases, including pulmonary hypertension [51], cancer [38], renal disease [41], and diabetic retinopathy [66]. There is extensive evidence that implicates PDGF in inhibiting SMC differentiation, and the extensive intracellular machinery (e.g., gene promoters and repressors) that enact its ability to shift SMC phenotypes from differentiated to synthetic/proliferative are well described [67]; for a review, see Ref. [36]. How the phenotypic states of a collection of SMCs within the medial wall, in turn, impact the mechanical stiffness of that tissue, which may further be influenced by regional NO levels, is less well understood and requires the type of multiscale modeling that we will focus on in subsequent sections of this chapter.

Thus far in this section, we have highlighted how small molecule signals (e.g., NO) and growth factors (e.g., PDGF) impact vascular adaptation, but we would be remiss to leave out the impact that ECM and its modifiers have on vascular growth and remodeling. While various extracellular proteins and glycoproteins (e.g., elastin, fibrillins, and fibulins: see Ref. [62]) provide a substrate for vascular cell assembly and stability, proteolytic enzymes such as the MMPs critically impact vessel homeostasis and adaptation by degrading the ECM (Fig. 2) and by mediating intercellular signaling (for review see Ref. [46]). The MMP family includes

collagenases, gelatinases, stromelysins, matrilysins, and membrane-type MMPs. ProMMPs are cleaved into active forms, which degrade ECM proteins, and their effects are balanced by tissue inhibitors of metalloproteinases (TIMPs) that prevent excessive proteolytic ECM degradation.

As noted in subsequent sections, MMP-2 and MMP-9 (gelatinases A and B) are upregulated during sustained hypertension and contribute to ECM reorganization, SMC proliferation and migration, and vascular hypertrophy in large vessels. Increased MMP-2 levels, in particular, have been associated with impaired NO-mediated vasorelaxation, arterial wall hypertrophy, and excessive collagen and elastin deposition. Therapeutic MMP inhibition with doxycycline has been proposed as a pharmacological strategy to attenuate SMC proliferation and hypertrophy during hypertension (for review see Ref. [15]) as well as the treatment of aneurysms. MMPs can also liberate and activate matrix-bound growth factors, such as TGF-beta [13], which may have opposing influences on SMC differentiation. Thus it is important to quantitatively assess these interactions with spatial and temporal resolution in order to resolve issues of therapeutic dose and timing.

### 3 Modeling Foundations and Current Models

#### *3.1 Continuum Biomechanics and Illustrative Vascular Models*

Continuum biomechanics has proven to be an important contributor to our understanding of physiology and pathophysiology as well as to the design of medical devices, biomaterials, and tissue engineered constructs. It is fundamental, for example, to many analyses of vascular biology and pathophysiology that are based on clinically available information such as blood pressure, local blood flow, and complex geometry [30]. Continuum biomechanics is founded upon five basic postulates: balance of mass, linear momentum, and energy as well as balance of angular momentum and the entropy inequality. Whereas the first three types of relations provide partial differential equations of motion, the last two provide important restrictions on the forms of the constitutive relations (i.e., descriptors of individual material behaviors). An underlying assumption is that one can compute at each macroscopic point (or location) and each instant a meaningful “continuum average” of properties or physical quantities of interest; a general guideline is that the continuum assumption is reasonable if the characteristic length scale of the microstructure is much less than the characteristic length scale of the physical problem. For example, continuum biomechanics can be equally applicable to studying an arterial wall (wherein diameters of collagen and elastic fibers are on the order of  $\mu\text{m}$  and overall vessel diameter is on the order of mm or cm) or an isolated cell (wherein diameters of cytoskeletal filaments are on the order of nm and overall cell dimensions are on the order of  $\mu\text{m}$ ).

To date, the two primary vascular applications of the continuum approach have been to compute pressure and velocity fields in blood flow (i.e., hemodynamics) and to compute stress and strain fields within the vascular wall (i.e., wall mechanics), each of which requires explicit solution of mass and linear momentum balance. Although cells cannot sense continuum metrics such as stress and strain, these quantities have proven useful in correlating mechanobiological responses by cells to diverse loads [31]. For example, simple parallel plate flow experiments demonstrate that ECs are very responsive to changes in wall shear stress, which is calculated using the continuum approach; simple organ culture experiments on straight segments of arteries and arterioles demonstrate that SMCs are very responsive to changes in pressure and extension, which induce intramural changes in stress and strain. Given the complexity of the microstructure of cells and tissues down to the level of molecular interactions, it is inconceivable that one would attempt to use a purely molecular dynamics simulation to study problems that manifest at a physiological or clinical scale. That is, continuum biomechanics is much more appropriate to study problems involving, for example, changes in the structural stiffness of the arterial wall in hypertension, the effects of evolving vascular diseases such as atherosclerosis or aneurysms, or the design of novel interventional devices such as intravascular stents, heart valves, or left ventricular assist devices.

Notwithstanding past successes, until recently continuum biomechanics had focused primarily on material behaviors at a particular time, not how they evolve. Moreover, most studies had assumed that the tissue (or cell) is materially uniform. Yet, all tissues are materially non-uniform, consisting of different types of cells and matrix that turnover, and so too cells consist of different organelles and cytoskeletal proteins that change over time. In an attempt to address these complexities, Humphrey and Rajagopal [33] proposed a Constrained Mixture Model (CMM) that allows one to track evolving changes in the properties, turnover rates, and natural (i.e., stress-free) configurations of individual structural constituents that comprise a tissue or cell. Computations have shown that this approach can capture salient features of diverse vascular adaptations and disease processes (cf. [6, 61]). Briefly, a CMM of arterial growth and remodeling consists of full mixture equations for mass balance plus a single equation for overall linear momentum balance that is solved for the net stress field. The associated two classes of constituents are: structurally insignificant but soluble constituents, such as vasoactive molecules, growth factors, cytokines, and proteases, and structurally significant but insoluble constituents, such as elastin, collagen, and muscle. Linear momentum balance is solved via a rule-of-mixtures constitutive relation for the structurally significant constituents. The need for but a single linear momentum equation stems from the assumption that negligible momentum exchanges exist between structurally significant constituents, which appear to deform together with the mixture. Because inertial loads are often negligible in the calculation of arterial wall stresses, even during transient loading, we assume further that structurally significant constituents experience quasi-static loading. The primary constitutive relations thus reduce to equations for the production and removal of structurally

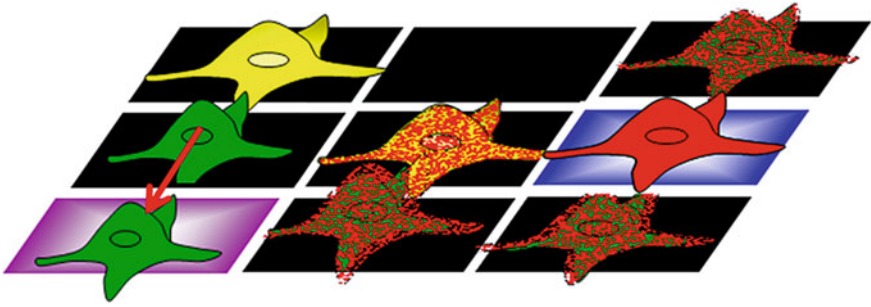
insignificant and significant constituents (the former via classical reaction–diffusion type relations) and stored-energy functions for the structurally significant constituents. Each of these relations must be formulated based on appropriate experimental data. For example, data reveal that NO not only causes vasodilatation (thus affecting the stress–strain behavior), it also affects the synthesis of collagen (production relation) and inhibits inflammation (which influences the removal relation).

### ***3.2 Agent Based Modeling and Illustrative Vascular-Specific Models***

Multi-cell biological phenomena, such as the assembly of cells into tissues in response to environmental cues, can be modeled using either continuum or discrete approaches. A primary focus of multi-cell modeling has been to quantify how patterns emerge in tissues and whether they arise from diffusible biochemical factors, mechanical forces, cell–cell interactions, cell–matrix interactions, or any combinations thereof. While most continuum-based models approximate individual cells as a series of similar units, discrete multi-cell models explicitly represent individual cells as distinct entities capable of exhibiting individual behaviors, which provides increased generality. Amongst the different approaches to discrete cell modeling, the most common are Agent-Based Models (ABMs), Cellular Potts Models (CPMs) [25], and statistical models. CPMs generalize an approach from statistical mechanics called the Ising model, and simulate biological cells by mapping them to domains on a lattice. Cell behaviors are described by effective energies and elastic constraints, and cellular dynamics, such as migration and cell shape changes, are guided by principles of energy minimization [16, 17, 34, 40, 70]. Statistical approaches, such as Monte Carlo simulations, model biological cells as discrete objects, and their behaviors are dictated by purely probabilistic rules [21].

#### **3.2.1 Agent Based Modeling**

ABMs represent a computational approach that has been used extensively in the social sciences and ecology [27], but only recently has it been employed in biomedical research to study multi-cell phenomena such as tumorigenesis [71], angiogenesis [44], inflammation [7], and arterial wall remodeling in hypertension [58]. This technique rests on the idea that local interactions between members of a population can result in complex higher-level emergent phenomena. The key components of an ABM include the agents themselves, their behaviors within their environment (i.e., simulation space), the rules that govern their behaviors, and the simulation inputs and outputs.



**Fig. 3** Components of an agent-based model. The grid of squares represents the simulation space, which can hold concentrations of extracellular factors, denoted here by colored squares. Agents/cells reside at discrete points on the grid, and are colored to represent cell phenotype or activity. Agents perform behaviors according to their rule set. For example, the *yellow* cell is currently static, with no outside influences, while the *green* cell is following a rule instructing it to migrate up a gradient of a chemokine (*purple*). A third cell (*red*) is affecting the phenotype of its neighbors through paracrine secretion of a growth factor (*blue*)

### 3.2.2 Agents

Agents are discrete entities that perform a defined set of behaviors according to a set of rules. Agents representing cells, for example, are programmed to exhibit biologically-relevant behaviors, including migration, proliferation, differentiation, and apoptosis [3–5, 43, 44] as shown in Fig. 3. Rules governing an agent’s decision to perform these behaviors may take into account, among other things, the agent’s past state history and factors present in the local simulated tissue environment; these rules can thus be stochastic or deterministic. Agents can detect biochemical and biomechanical stimuli in their environment and respond by exhibiting a particular behavior. Agents can also affect their environment by secreting diffusible growth factors (e.g., PDGF-BB) or by producing ECM and its modifiers, such as collagen or MMP-9. Finally, agents can interact with one another. For example, neighboring agents with engaged cadherins can interact physically much in the same way as neighboring cells in a tissue would interact—by signaling directly to one another, transmitting a mechanical force, and so forth. All of these actions and interactions will impact an agent’s state, and the ABM can record the state histories of each agent at each time step, facilitating individual cell lineage tracking. To simulate more complex phenomena, multiple cell types can be represented using multiple types of agents. For example, an ABM modeling the progression of an atherosclerotic plaque might contain agents that represent individual ECs, SMCs, macrophages, foam cells, or fibroblasts. The aggregate of agent behaviors and interactions over space and time produces emergent phenomena that could not be predicted by modeling a single cell or using models making a continuum assumption.

### 3.2.3 Rules

Agent-specific actions and interactions in an ABM are ultimately dictated by the rule set. These rules can be theoretically or empirically based and can be deterministic or stochastic. An example of a theoretically based rule is the use of Fick's second law to describe the diffusion of a growth factor that affects other cells [44]. An example of an empirically based rule is a dose–response curve describing the speed of cellular migration as a function of chemokine (e.g., IL-8) concentration. Stochastic rules rely on probability distributions and are important when an agent state does not explicitly dictate a behavior, but affects the likelihood of such a behavior. Rules are most often derived or estimated from the literature; the organization and presentation of these rules is facilitated by a table or flow chart accompanying the ABM (cf. [7]). Rule sets can vary from fewer than ten rules [26] to as many as 200 [7]. The composition, content, and accuracy of a rule set have a profound impact on the output of an ABM. Slight modifications to a single rule can dramatically alter the output of even the simplest ABM. Therefore, in designing, constructing, and implementing ABM rule sets, caution is urged to ensure that the rules are accurate, non-redundant, and necessary.

The validity of a rule set can be checked by contrasting model outputs against experimental data [39, 44, 55], and by performing a sensitivity analysis, where rules are systematically removed or adjusted incrementally to determine their contribution to the overall ABM output [26]. Because the outputs of an ABM are highly dependent on empirical rules, it is necessary to couple models with experiments at all stages of model development and to validate an ABM's rule set by performing iterative *in silico* and *in vivo/in vitro* experimentation. For a review of the integration of experimental data with ABMs, see Ref. [57], and for a method of assessing the quality of a model's rule set, see Ref. [58].

### 3.2.4 Inputs and Outputs

Most biological ABMs simplify tissue geometry by simulating cell behaviors in a quasi-two-dimensional simulation space that reduces model complexity and speeds up simulations. For example, one can use a one-cell thick axial slice of vessel to model arterial adaptations to hypertension [58]. This simplification enabled measurement of vascular wall thickness and cellularity and was sufficient to enable calculation of the concentration and diffusion of extracellular proteins as well as to facilitate direct comparisons with experimental data. An ABM simulation space can have closed [39], open [7], or periodic boundary conditions [53], and the positioning and state assignments for the agents at the start of a simulation are specified by initial conditions that are frequently derived from microscopic images obtained at a starting time [7, 39]. Setting initial conditions in this way enables direct comparisons with the experimental data at later time-points for model validation. The initial agent states are assigned based on empirical observations (e.g., histology) that describe baseline conditions for agent states. The time

steps of biological ABMs can span a range of scales, from milliseconds to hours [14, 44], and simulation times can span years [1].

Outputs of ABMs include the spatial arrangements of agents within their simulation space, their internal state, and the state of the environment. Spatial patterns of agent organization may emerge during the simulation, and these can be analyzed quantitatively. For example, in an ABM simulating angiogenesis, the pattern of new microvessel growth can be assessed by measuring the new vascular length, counting branch points of vessel trees, and quantifying the number of new capillary sprouts that have developed over the course of the simulation time window. Analyzing agent patterns using metrics that are also used to quantify biological phenomena experimentally facilitates direct comparison of ABM predictions to experimental data, which enables rigorous model validation. Because ABMs generalize intracellular processes, but are more fine-grained (discrete) than is suitable for continuum analysis, they are uniquely suited to bridging disparate biological scales.

### ***3.3 Signaling Pathways and Illustrative Vascular-Specific Models***

Intracellular signaling pathways govern basic cell functions and allow cells to adapt to their microenvironments. These signaling pathways consist of a complex network of interacting molecules that give rise to a diverse range of cell functions such as proliferation and differentiation. Often, an extracellular ligand binds to a cell-surface receptor and triggers a cascade of intracellular interactions between signaling molecules and second messengers that ultimately results in a change in transcriptional activity, metabolism, or other regulatory function.

Because network elements of signaling pathways often overlap, the causal relationship between input and output is not always explained by a linear series of events. Furthermore, network motifs, such as positive and negative feedback loops, make it difficult to deduce the relationships between the network elements solely by intuition [2]. To better study and understand intracellular signaling pathways and networks, a combination of experimental and mathematical approaches have been used to disentangle the functions of the highly interconnected components. Mathematical and computational Intracellular Signaling Models (ISM) are used to contextualize experimental data and predict possible emergent behaviors that are difficult to realize by experimentation alone. Through cycles of model refinement and experimental validation, one can begin to understand and probe the complexities of the signaling network and make predictions about how specific cellular functions arise.

These computational models predict dynamic behaviors of biochemical reactions by using mathematical relations to describe the underlying molecular interactions. Traditionally, ordinary differential equations (ODEs) are used to model



the concentration profiles of the different signaling molecules over time in response to a stimulus. Mass action and Michaelis–Menten kinetics are common ways to represent the reaction kinetics. These approaches have been used to demonstrate how even relatively small and focused modules can exhibit emergent behavior through feedback mechanisms [11]. If information about the spatial distributions of the molecules is desired, partial differential equations (PDEs) are used. ODEs and PDEs can then be implemented deterministically or stochastically. Deterministic systems have no element of randomness while stochastic systems incorporate probabilities in the evolution of the system output over time. The choice of how the model is formulated depends upon how the modeler views the system and the biological question being answered.

While modeling of intracellular signaling networks pertaining to blood vessels is still a relatively young field, an increasing number of computational models are being developed to study vascular function. To date, a few aspects of vascular intracellular signaling have been prominently modeled. Proliferation of ECs and the formation of vessels is one such area. For example, several intracellular signaling network models have been developed to study vessel formation in the context of vasculogenesis during embryonic development and angiogenesis in both physiologic and pathophysiological cases [37, 45, 52]. In terms of particular signaling pathways, those involving NO and calcium have received much attention in recent computational models [60, 65]. We will summarize some of the published models of vascular signaling networks in the following paragraphs and highlight the new understanding they produced.

As noted above, among other functions, NO is a key signaling molecule that regulates tissue-level vasodilation by affecting cell-level (SMC) contraction. One group modeled the NO/cGMP signaling pathway in vascular SMCs and reproduced NO/cGMP-induced smooth muscle relaxation effects, such as intracellular  $\text{Ca}^{2+}$  concentration reduction and  $\text{Ca}^{2+}$  desensitization of myosin phosphorylation and force generation [65]. The authors of this model proposed a cGMP feedback-controlled soluble guanylate cyclase (sGC) decay from its activated to its basal form and predicted that the intermediate form of sGC is the dominant steady-state form of sGC under physiological NO stimulation. This model thus suggested that the sGC desensitization by cGMP feedback may limit cGMP production over a wide range of NO concentration, which may contribute to the robustness of the response of vascular SMCs to small perturbations in NO. The different mathematical models used to investigate the role of NO in microcirculatory physiology have been reviewed in [60], and one of the overarching themes is that intracellular signaling models can provide valuable insights into roles of NO in physiology, especially because experimentally measuring tracer amounts and signaling events of NO in biological tissue with the appropriate spatial and temporal resolutions is difficult.

Calcium signaling is largely coupled to NO signaling. In the arterioles, SMCs and ECs are coupled via the exchange of  $\text{Ca}^{2+}$  along with other ions and the paracrine diffusion of NO. The vascular response to the nonlinear interactions of subcellular components and processes including  $\text{Ca}^{2+}$  signaling have been studied



in rat mesenteric arterioles via mathematical modeling [35]. This ODE model predicted that in the rat mesenteric arterioles, ECs exert a stabilizing effect on intracellular vascular SMC  $\text{Ca}^{2+}$  oscillations, which synchronize with oscillations in vessel tone to cause vasomotion. This stabilization allows  $\text{Ca}^{2+}$  oscillations to be maintained over a wider range of agonist concentrations. Models related to intracellular signaling include those that simulate the dynamics of ionic flows across the membrane. One group modeled the dynamics of the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger (NCX) in vascular SMCs; this exchanger regulates the reloading of the sarcoplasmic reticulum and the maintenance of  $\text{Ca}^{2+}$  oscillations in activated SMCs [23]. Although this model is not of an actual signaling network, it gives insights into the mechanism of the coupling between  $\text{Na}^+$  entry via TRPC6 (a non-specific cation channel) and the NCX. This implicates the concentration of  $\text{Ca}^{2+}$  in the vascular SMC, which affects the signaling pathways involving  $\text{Ca}^{2+}$ . The model incorporates a stochastic element to simulate the movement of single  $\text{Na}^+$  ions in the nanospace between the plasma membrane and the sarcoplasmic reticulum. This model predicted that in order to have a  $\text{Na}^+$  concentration transiently elevated in the plasma membrane/sarcoplasmic reticulum nanospace, there must be physical obstructions to  $\text{Na}^+$  motion, which form a relatively impermeable barrier around the TRPC6 channel. NCX must also be localized near TRPC6 within such barrier in order to sense the high  $\text{Na}^+$  concentration, reverse, and allow  $\text{Ca}^{2+}$  into the sarcoplasmic reticulum. As the details of individual intracellular signaling pathways become better understood, models can be evolved to incorporate additional pathways such that they combine to form an interconnected signaling network that increasingly describes the physiological system with more accuracy.

### ***3.4 Limitations of Singular Modeling Approaches***

#### **3.4.1 CMM Limitations**

Continuum models have proved very useful in vascular research, including helping to reveal the existence of residual stresses and their effects on the transmural distribution of stress that led to a fundamental mechanobiological hypothesis [32]. Continuum models also continue to be very successful in explaining and predicting a wide variety of nontrivial aspects of vascular physiology and pathophysiology, including the adaptation of arteries to sustained changes in blood pressure or flow as well as the rupture of aneurysms. Nevertheless, limitations remain. For example, the continuum approach assumes material is distributed continuously over particular length scales, which can mask specific mechanisms of mechanotransduction that result from cell–matrix interactions at discrete sites (e.g., focal adhesions) but otherwise help to drive overall tissue-level adaptations. Current models also do not account for the details of particular matrix–matrix interactions, including interactions between collagen fibers and proteoglycans. Perhaps most

importantly, however, CMMs cannot exploit directly the growing knowledge-base of molecular vascular biology.

### 3.4.2 ABM Limitations

Agent-based models can explicitly include complex cell–cell interactions, but are limited when it comes to modeling multiscale phenomena. For example, current ABMs do not satisfy some of the important constraints on vascular behavior that stem from classical physics (e.g., conservation of linear momentum). Simulating whole tissues would also require an extremely large number of individual agents, which quickly becomes prohibitive computationally, particularly for complex rule-sets that may include stochastic rules. While stochastic rules can produce a population of results that more closely resembles actual experimental data, including stochasticity necessitates an even larger number of model runs to converge on an average output. Finally, single-scale, cell-level ABMs treat the agent as a black box; intracellular interactions are often included implicitly when rules are derived from cell-level experiments. When intracellular processes are simple, this is less of a problem, but as intracellular interactions grow more complex, experimental approaches to rule development can become intractable. Of course, as with any model, the ABM rule-set is only as good as the data from which it is derived. Often the kind of data needed to develop rules for cellular behaviors with complex multifactorial inputs are not available in the literature.

### 3.4.3 ISM Limitations

Models of intracellular signaling networks are useful tools in predicting the effects of signal transduction in a cell in response to a stimulus. These predictions are made at the cellular level, and may require coupling with higher level models to infer tissue or organ level function. For example,  $\text{Ca}^{2+}$  signaling in vascular smooth muscle cells is studied to make predictions about vasomotion in blood vessels. Vasomotion requires the synchronization of oscillations in the concentration of  $\text{Ca}^{2+}$  in a large group of vascular smooth muscle cells, and gap junctions are believed to play an important role in this process [18]. Hence, elements of cell–cell interactions often need to be coupled to an ISM to convincingly bridge the gap between single cell and tissue level predictions. Lastly, as with the other types of mathematical models, determination of the scope and complexity of an ISM during the developmental stages is not a trivial task. In some cases, the paucity and confidence of relevant experimental data to be used as model parameters can directly limit the scope and usefulness of an ISM.

## 4 Linking Intracellular, Cellular, and Tissue Level Models

### 4.1 *An Integrative Approach*

Single-scale models of the types described above all have their own unique advantages but also limitations. In vivo changes in vascular wall structure depend critically on interactions at all scale levels, from intracellular signaling to whole vessel biomechanics. A more complete understanding of the responses of blood vessels to changes in hemodynamics requires detail at the cellular level that CMMs alone cannot provide, while many of the functions of SMCs (such as proliferation and collagen production) result from the integration of so many signals that experiments to develop rules for a single-scale, cellular level ABM, are not feasible. Such an integration of signals could be carried out by an ISM, however. A multiscale, multi-model approach is thus in order, which promises to build on the strengths of each type of model while compensating for limitations by offloading them to another model better suited for those specific types of tasks.

Coupling models at different spatial and temporal scales brings forth a whole new set of challenges, however. In the next two sections, we focus on challenges unique to each coupling (intracellular to cellular-level, and cellular-level to tissue-level). We suggest ideas to consider when attempting this approach, and provide examples from the literature where these techniques have been successful.

### 4.2 *Coupling Intracellular Signaling Models with Agent Based Models*

Rather than building ABMs whose rules treat the cell as a black box, which provides specific outputs and behaviors given certain combinations of inputs, it is possible (experimental data permitting) to couple an ABM with an ISM. In this case, each agent monitors concentrations of extracellular signaling molecules and runs its own ISM to decide on a particular behavior, including amounts of proteins to produce. Such a coupling could thereby enable more fine-grained simulations that more closely replicate in vivo intracellular processes.

One of the areas where cellular-level ABMs can break down is in the combination of multiple influences on a single output. For example, both TGF- $\beta$  and PDGF-AB influence collagen production by SMCs [58]. Each biomolecule's influences can be tested experimentally by varying concentrations applied in culture, but mapping out responses by SMCs to combinations of growth factors increases the complexity of the experiment exponentially. Indeed, addition of a third or fourth growth factor could make the experiment intractable. Hence, these kinds of experiments are rarely performed in vitro even though they are exactly the kind needed to inform a cell-level ABM that treats the cell as a black box. For example, development of a rule for the combined influences of growth factors on

collagen production currently requires extrapolation from a few data points or parameterization of the ABM [58]. Conversely, if the appropriate intracellular signaling pathways were well understood, experimentally inferred rules could be improved by coupling the determination of collagen production to an ISM resulting in more physiological model output.

The scenario described above, however, would require each agent to run its own ISM or to query a single ISM running separately at every timestep. As the number of agents increases, this could become computationally expensive. Therefore, it is advisable to carefully select which rules or outputs should receive this treatment. A thorough sensitivity analysis of the ABM assists in this process by determining which cellular outputs are most important to model predictions. In contrast, another way to approach this coupling would be to pre-calculate outputs of the ISM within physiological ranges of the input parameters and then store results in a look-up table to be accessed by agents during the ABM simulation.

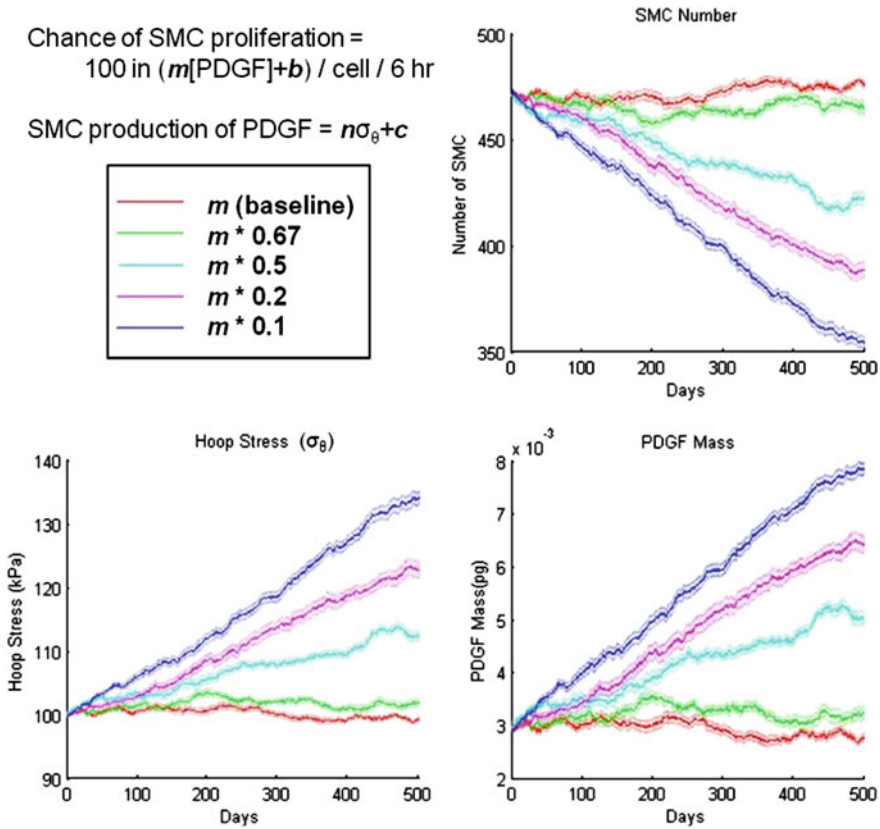
Other things to consider when determining how to couple cell-level with intracellular models is that temporal and spatial scales may differ by orders of magnitude. While the ideal time-step for an ABM may be on the order of minutes to days (e.g., depending on whether one is simulating cell migration or proliferation), intracellular protein interactions may occur on the order of nanoseconds to seconds. The majority of vascular models using some form of ABM-ISM integration to date have been in the field of angiogenesis. For example, Bauer et al. [8] developed a model of tumor induced angiogenesis using a cellular Potts model. In order to understand contributions of cadherins and ECM binding integrins to VEGF signaling, and the associated decision of a cell to migrate, proliferate, or apoptose, the authors went on to develop a Boolean network model incorporating the crosstalk between these three intracellular signaling pathways [9]. This model could potentially be used in conjunction with the CPM to dictate cellular behavior during angiogenesis. Likewise, Scianna [52] developed a hybrid approach coupling a CPM of vasculogenesis with an ISM using reaction-diffusion equations to couple VEGF signaling, arachidonic acid, and NO with calcium entry into the cell. These reactions occur with ten diffusion time steps per main time step.

The issue of very small time-scales for the ISM is much more easily addressed than the converse: when the signaling pathway being modeled contains transcription and translation, critical functions may occur over multiple ABM time-steps. In this case, the ISM might need to be capable of integrating changes in growth factor concentrations as a cell migrates, which may influence protein outputs 30 or more ABM time-steps later. While this has been addressed in previous ABMs by assuming that changes in protein levels happen instantaneously, or within one time-step [7], this may not always be possible when predicting sensitive outputs that require sufficient physiological detail. To our knowledge, this problem has not yet been satisfactorily solved and is something that needs to be seriously considered in the design phase of any integrated model.

### ***4.3 Coupling Agent Based Models with Constrained Mixture Models***

In this section we focus on coupling an ABM with a CMM, but the overall procedure can be applied to the coupling of many types of models. Any model is only as good as the data upon which it is built. Hence, we suggest that the first step in a multistep process of coupling different models should be to substantiate the goodness of the published data used to build both models. This can be done, in part, by evaluating the credibility of each data set based on four categories: (1) agreement with other published findings, (2) physiological conditions (e.g., in vitro versus in vivo), (3) appropriateness to the computational model (this metric assesses how well the data match the particular situation, that is, same cell type, organ system, species, environmental condition, and so forth) and (4) data precision (e.g., data measured directly and quantified numerically are better than extrapolated or theoretical); please see Ref. [58] for more details. After performing this data assessment one can then use the highest scored findings to update or create a data-driven computational model with more confidence.

The second step involves further model verification through stability and parameter sensitivity analyses. After the governing equations, parameters, and outputs have been defined for each model, one should confirm that the model is stable over known situations. For example, constituents within the vascular wall are synthesized and turned over at different rates; collagen may be secreted by the cell in less than an hour and has a half-life of  $\sim 30\text{--}70$  days, whereas elastin is primarily produced during the perinatal period and remains for the majority of one's lifetime. Despite these changes, the average geometry of a healthy, mature artery remains fairly constant over long periods. Therefore, it is expected that, under homeostatic conditions, the ABM and CMM should predict no net change in geometry, microstructure, mechanical properties, or biological response over such periods. Moreover, one should confirm that the model can capture acute reactions to transient perturbations. For example, a 10 % increase in pressure over a few hours can lead to transient spikes in growth factor production and yet no net changes in collagen or SMC content. In order to assess the sensitivity of each model to parameters that influence production and removal rates, it is important to conduct a one-dimensional, and if possible, a two-dimensional sensitivity analysis. A one-dimensional analysis will reveal to what degree a single parameter can be increased or decreased before outputs diverge from what is physiologically expected. For example, in a recently reported ABM, the production of new SMCs depended in part on a gain-type parameter multiplied by the concentration of PDGF; if this parameter increased, then SMC content increased and so too wall thickness, which could decrease the circumferential stress (Fig. 4). Yet, PDGF production is a function of circumferential stress, thus less PDGF should be



**Fig. 4** Sensitivity of the number of smooth muscle cells (SMC), value of hoop stress and concentration of PDGF to changes of the  $m$  parameter in the chance of SMC proliferation function. Note the interdependency between PDGF, hoop stress and number of SMC. Outputs are able to obtain a new equilibrium to small changes of the  $m$  parameter

produced as the wall thickens (Fig. 4). Ensuring proper responses to simple perturbations, as illustrated by this example, can help identify appropriate bounds to place on free parameters.

Finally, note that multi-cell ABM rules are often based on cell level in vitro experiments, which can be well controlled but do not always have direct physiologic relevance. CMMs are typically based on either in vitro or in vivo tissue-level experiments, which can have considerable physiological relevance but often little control of the inputs sensed by the cells (e.g., humoral in addition to hemodynamic). Because each data set will be inherently limited, some advantage can be gained by ensuring that models of the same processes based on very different data should yield the same result [29]. The third step, therefore, is to enforce congruency across two (or potentially more) scales through parameter

refinement. This process allows one to refine the objectively bounded model parameters in an attempt to minimize the error between common metrics between both models for simple situations. This step is imperative to synchronize both models across scales and to yield predictions that are closer to in vivo observations. This is accomplished not by compromising the strengths of either model, but by tuning each model so that it is influenced by the strengths of the other. For example, the ABM and CMM both have the ability to predict the amounts of collagen and smooth muscle under simple situations; therefore by instituting a genetic algorithm, or any preferred error minimization technique, the difference between these common metrics, at each time point, can be minimized by varying the set of parameters after each full simulation.

## 5 Future Directions

### 5.1 *Potential Multiscale Model*

A compelling goal in developing multiscale models is to extend the models to both higher and lower levels of scale, with the motivation being that with every added level of scale, one gains even greater flexibility with regards to hypothesis testing, achieving biological relevance, and incorporation of disparate data sets. Starting with the multiscale CMM-ABM, a natural extension is to conjoin an intracellular signaling model (ISM) that can simulate events on a much shorter timescale and account for phenomena within the cell that ultimately impact cell behaviors and outcomes at the tissue level. We have begun to conceptualize a three-tiered multiscale model (ISM-ABM-CMM) of vascular growth and remodeling in the arterial wall (Fig. 5), and this section will briefly summarize some of the enabling components.

The relevant biology of vascular growth and remodeling during human disease occurs over years; however, biological phenomena contributing to disease progression over this timeframe occur across incremental time steps that range from seconds to weeks, depending on the spatial scale. Specifically, signaling events in the ISM have a timeframe on the order of seconds to minutes, multi-cell phenomena in the ABM have a timeframe of minutes to hours, and tissue-level continuum phenomena in the CMM have a timeframe of hours to days or months. Therefore, in the construction of the multiscale model we envision a temporal decomposition strategy to couple across scales (Box 1).

### **Box 1. Temporal decomposition strategy for a three-tiered multiscale model**

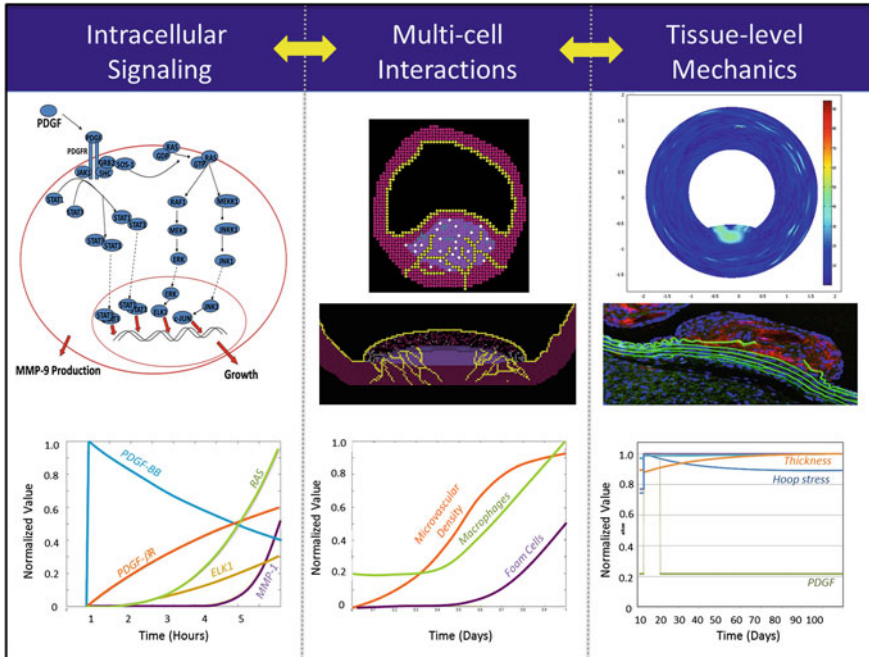
**Intracellular to multicellular time interfacing:** Each time step of the ABM will represent 1 h, and multi-cell level behaviors will occur on this time-scale. For example, SMCs will proliferate over 8–24 h [44] or 8–24 time steps in the ABM, while growth factor production will occur over 12–24 h (or time steps) [44]. When the ABM calls the ISM (every 24 h for 120 simulated days), where biological events will be simulated on the order of minutes, we will institute a time-delay, or essentially “pause” the ABM, while the intracellular events are computed by the ISM.

**Multicellular to tissue-level time interfacing:** Each time step of the CMM will represent 1 week, and tissue-level behaviors will occur on this time-scale. For example, the strain state of the artery wall will change over the course of 1–2 weeks (depending on vessel geometry and composition alterations during this time window). Therefore, to account for these changes on a time window that is biologically realistic, the ABM will call the CMM every week (i.e., every CMM time step) in order to recompute and update tissue-level changes that occurred during this window. Thus, in the ABM, every 7 days (or 168 time steps in the ABM), the ABM module will be “paused” in order to run the CMM, whose output (computation of the mechanical state of the media) will be imported back into the ABM.

Another hurdle to achieving a unified three-tiered multiscale model is defining an appropriate level of resolution and abstraction for the relevant spatial scales such that information at one level can be mapped to higher and/or lower levels of biological scale. We have previously devised a method for coupling spatial scales between an ABM and ISM [49]. In that ABM module, we simulated individual biological cells using nine coupled agents that represented different cytoplasmic and membrane compartments within each simulated cell. In this way, we could simulate the differential behaviors of the leading versus trailing edge of a cell as it migrated across a two-dimensional substrate. Intracellular signaling events were simulated in the ISM and distributed equally to the nine agents comprising each cell, but one could envision partitioning or compartmentalizing certain reactions within spatially confined intracellular regions that would map directly to the discretized, multi-agent cells within the ABM. We have similarly devised a method for comparing spatial scales between an ABM and CMM [29]. In this case, the ABM consisted of layers of cells whereas the CMM consisted of a homogenized structural wall. Data from the ABM could thus be averaged radially and applied to the CMM at each time of interest.

We envision that a three-tiered, multiscale ISM-ABM-CMM will be united by an umbrella program implemented in Java<sup>TM</sup>. This program will run the ISM, ABM, and CMM modules in parallel, while transferring information between each pair of modules. The CMM and ISM are implemented in Matlab<sup>®</sup>, and the ABM is implemented in Netlogo [63]. We have opted to use these modeling software





**Fig. 5** Illustration of a possible approach to couple intracellular signaling models (ISM), agent based models (ABM), and constrained mixture models (CMM) to enhance computations of vascular behaviors

programs because they are well established, readily available, and easy to learn, which, importantly, will facilitate the sharing of our multiscale model. Matlab is a general-purpose simulation tool, and Netlogo, written in Java<sup>TM</sup>, is freely downloadable from <http://ccl.northwestern.edu/netlogo/> and is the most widely used agent-based modeling software [63]. Our group has previously published a multiscale model that unites ISM with ABM using a Java<sup>TM</sup>-based umbrella program to run an ISM and an ABM in parallel [49], for the study of tissue morphogenesis during embryonic development. The umbrella program will interface the ISM, ABM, and CMM through the use of text files that can be imported and exported by both Matlab<sup>®</sup> and Netlogo, which will store inputs and outputs of each module and be used to communicate predictions from one level of scale to the next. Netlogo extensions are capable of running Java<sup>TM</sup> code, and the Java<sup>TM</sup> code will open and run Matlab.

Model validation is an important check to confirm the model is accurate and stable. Box 2 describes a possible strategy for validating a three-tiered multiscale model.

### **Box 2. Validation Strategy ISM-ABM-CMM**

The multiscale model can be validated by first validating each of the modules (ISM, ABM, and CMM) individually (Steps 1 and 2), and then validating the integrity of the unified multiscale model (Step 3):

**Step 1:** Disconnect each of the modules (ISM, ABM, and CMM) from one another and compare outputs from each module to independent experimental data collected (or reported in the literature) at that level of scale. For example, to validate the ISM, one can perform a set of simulations to acquire an *in silico* dose–response curve relating SMC proliferation rates to PDGF-BB concentration. Then compare this predicted dose–response curve to experimentally measured proliferation rates from PDGF-BB dose–response studies. This will ensure that the internal structure of the ISM accurately reproduces SMC proliferation in response to PDGF-BB.

**Step 2:** Disconnect each of the modules (ISM, ABM, and CMM) from one another and compare those outputs that each module has in common with one another. This will ensure that the modules are internally consistent with one another. For example, both the CMM and the ABM will predict the thickness of an atherosclerotic plaque, but it is important to check that the predictions from both modules are congruent (and minimize the residual differences between ABM and CMM predictions), given that the parameters and “rules” governing the ABM and CMM will be derived from different sources (Fig. 5).

**Step 3:** Validate the unified multiscale model by comparing its outputs (i.e., outputs generated by the *integrated* ISM, ABM, and CMM) to independent experimental data. Quantitative predictions of the multiscale model at time  $t > t_0$  can be compared with identical outcome metrics collected from the quantitative analyses of hypertensive patients or ApoE  $-/-$  plaques. Agreement between prediction and experiment will suggest that the multiscale model is valid for that range of parameters

The hope in pursuing this type of multiscale modeling is that one day we will be able to confidently theorize about new drug or knock-out treatments and cause-effect relationships. For example, if the angiotensin II (ANG-II) receptor type II (AT-2) is blocked, ANG-II binds to the type I receptor (AT-1) of ECs. Binding of AT-1 activates the tyrosine kinase and downstream proteins (mitogen-activated protein kinase (MAPK), Janus kinase (JNK), and signal transducer and activator of transcription (STAT)) leading to increased intracellular calcium, activation of the L-type calcium channel, and consequently arterial constriction. Activation of MAPK also stimulates fibroblast and SMC migration and proliferation via synthesis of platelet derived growth factor and tissue growth factor- $\beta$ . These growth factors as well as increased aldosterone all serve to facilitate extracellular matrix production in a particular collagen, which leads to increased wall stiffening or pulse wave velocity. Stiffened arteries not only require a larger pressure to distend; flow propagation is impaired due to inadequate elastic recoil. Thus over time the

increased load on the heart causes left ventricle hypertrophy and left ventricle failure. Alternatively, we can theorize about “top-down” effects. For example, how does mechanical shear force experienced by the ECs impact NO signaling and PDGF expression, which in turn affect SMCs and overall wall mechanics? Creating models to predict these types of outcomes will save time, money, and potentially lives.

Of course, the aspiration to unite ISM-ABM-CMM is met with considerable challenges, not the least of which is the requirement for additional computing power. Assuming the technical challenges can be overcome by advances in computing (e.g., parallelization, grid computing, and cloud computing), one must address the conceptual challenges in multiscale model design. The final section of this chapter will delve deeper into these challenges and suggest opportunities for innovation in multiscale modeling.

## 5.2 Challenges

As noted by the 1998 Bioengineering Consortium (BECON) Report of the U.S. National Institutes of Health,

The success of reductionist and molecular approaches in modern medical science has led to an explosion of information, but progress in integrating information has lagged ... Mathematical models provide a rational approach for integrating this ocean of data, as well as providing deep insight into biological processes.

Whereas the need remains to develop more robust and faithful models at all scales (macro, micro, nano), we submit that there is a pressing need to develop approaches that integrate such models across diverse scales. Indeed, anticipating the challenges of multiscale modeling should influence the development of models at each scale for they will need to interface with the other models. Toward this end, we suggest here the following particular challenges that deserve our immediate attention.

There are several computational languages used to run numerical analysis (e.g., Matlab, Maple, Mathematica, Java Virtual Machine, FORTRAN, and C++). Thus, a logistical challenge may arise when models, at different scales, are programmed with different languages. We proposed herein using text files as inputs/output because all our modeling platforms can read and write text files, however this process is time consuming and cumbersome. Therefore finding patches or proper interfaces between multiple numerical analysis software remains a challenge. In addition, iterative simulations may take days to complete and require considerable memory on a personal computer. Consequently large-capacity databases and fast processors/parallel systems may be required to render the computational process tractable. After a multiscale program is completed, finding ways to distill and partition model findings into digestible chunks that are easy to disseminate and publish may be a challenge.

In addition to computational challenges, there are also many conceptual challenges. For example, events at the tissue level may depend on past events and take hours to days to occur, while at the intracellular level processes may occur in a fraction of a second. In addition to integrating across temporal scales, integrating across spatial scales (i.e., 2D versus 3D) may require further dispensation. One of the major challenges remains in simplifying the complex system due to gaps in our understanding or in an attempt to not over constrain the model. Where should one start, and how is each decision justified? Another challenge of integrating multiple discrete models is deciding what information to pass back and forth. Each model may have interdependency within itself, thus passing a concentration or stress value negates any feedback mechanisms the model had related to these parameters. Therefore, integrating models that rely on values from one another is a challenge.

Biological adaptation and variability are difficult to capture in a universal mathematical model. How biological systems change in time is what the models presented herein try to account for, but some adaptations are unpredictable. For example, natural effects (due to ageing, hormonal life cycles, ones genetic makeup, even what division cycle cells in the body are on) and external effects (due to accidents, smoking, exercise, eating habits, radiation, etc.) may alter how the general process works. Therefore, if the response of one patient or system could be very different than another, are the models unique to the patient? How general should the models be? Of course we are currently limited by our technology to measure and characterize the interactions of phenomenon of biological systems. Generally speaking, like the Heisenberg uncertainty principle, to augment our knowledge of, say, the rate of growth factor production may come at the cost of compromising physiological conditions.

Nevertheless, we feel that complex system modeling in biology is the key to developing new drugs and therapies over the next 50 years; as such there are educational needs that should be met. Having more undergraduate courses that deal with complex systems analysis in biology will equip more students with the fundamental skills. More graduate courses on the theory of modeling vascular adaptation, and biological adaptation in general, will allow for specialization and additional improvements. Having more graduate programs and/or cross-degree or dual-degree Ph.D. programs that are designed to treat high-throughput data in the context of in vivo function and quantitative modeling is needed. In addition, continued changes in academic culture that recognize the value of collaboration and teamwork on large complex systems will facilitate more advanced models. We are encouraged to hear that in April 2012, NSF and NIH jointly launched a “Core Techniques and Technologies for Advancing Big Data Science and Engineering (BIGDATA)” initiative. The need for a means to manage, analyze, visualize, and extract data from diverse, distributed data sets has been recognized. If successful, having this wealth of ordered data at our fingertips will only help to update and improve the rules and relations of multiscale modeling.

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