Critical Reviews™ in Biomedical Engineering, 41(2):91–123 (2013)

Manipulating the Microvasculature and Its Microenvironment

Laxminarayanan Krishnan,[1] Carlos C. Chang,[1] Sara S Nunes,[2] Stuart K. Williams,[1] Jeffrey A. Weiss,[3] & James B. Hoying[1]

[1]Cardiovascular Innovation Institute, Louisville, KY; [2]Division of Experimental Therapeutics, Toronto General Research Institute, Toronto General Hospital, Toronto, Ontario, Canada; [3]Department of Bioengineering, University of Utah, Salt Lake City, UT

*Address all correspondence to: Laxminarayanan Krishnan, PhD, Parker H. Petit Institute for Bioengineering and Biosciences, 315 Ferst Dr NW., Atlanta, GA 30332; laxmi.krishnan@ibb.gatech.edu.

ABSTRACT: The microvasculature is a dynamic cellular system necessary for tissue health and function. Therapeutic strategies that target the microvasculature are expanding and evolving, including those promoting angiogenesis and microvascular expansion. When considering how to manipulate angiogenesis, either as part of a tissue construction approach or a therapy to improve tissue blood flow, it is important to know the microenvironmental factors that regulate and direct neovessel sprouting and growth. Much is known concerning both diffusible and matrix-bound angiogenic factors, which stimulate and guide angiogenic activity. How the other aspects of the extravascular microenvironment, including tissue biomechanics and structure, influence new vessel formation is less well known. Recent research, however, is providing new insights into these mechanisms and demonstrating that the extent and character of angiogenesis (and the resulting new microcirculation) is significantly affected. These observations and the resulting implications with respect to tissue construction and microvascular therapy are addressed.

KEY WORDS: angiogenesis, microvessels, microvascular orientation, microvascular remodeling, microvessel guidance, three-dimensional (3D) vascular constructs, matrix mechanics

I. INTRODUCTION

Numerous efforts have been underway to assemble tissue-engineered solutions for clinical use, with several under development for wound repair or the repair and/or replacement of cardiac muscle, skeletal muscle, spinal cord, tendons, cartilage, skin, and bladder.[1–5] A majority of the successful efforts have involved thin, sheet-like tissue constructs, such as regenerative patches,[6] heart valves,[7] and replacement skin,[8] likely because of the limits of diffusion in cell-seeded constructs.[9] In most implants, nutritional maintenance relies on diffusion from the implant site until the implant is vascularized by the circulation of the host.[10,11] This is more difficult to realize in larger tissues in which tissue volumes, even as thin as 300 μm, can develop necrotic cores after 1 week of implantation.[12,13] Strategies for functionally supporting larger constructs have included the implantation of artificial constructs in areas of high vascularity,[14] addition of proangiogenic factors,[15] or seeding with supportive cells that produced chemotactic signals for recruiting host cells and microvessels.[16–18] Additional strategies have focused on prevascularizing the tissue space and/or tissue construct through the incorporation of either intact microvessels[19–21] or cultured vascular cells.[16,22–24] These approaches highlight the broad goal of establishing a functional associated vascular supply as a means to support the health and functionality of the implanted tissues.

The primary focus of inducing or constructing a vascular supply has been to form new microvessels; vascularization is increasingly being viewed as one of the primary challenges in tissue engineering of large 3-dimensional (3D) scaffolds.[25,26] However, an effective microcirculation depends on the density of the microvessels, the organization of these microvessels within a network, and the morphology and functional character of the microvessels—in other words, the formation of a microvascular network. A single capillary brings blood into close proximity of an area that is a few cell layers thick. A network of capillaries and other microvessels is

1040-8401/13/$35.00

necessary to provide sufficient exchange to support an entire tissue bed.[21,27] Therefore, unlike conduit vessels, where a single vessel meets the primary purpose (i.e., the bulk transport of blood), it is the microvessel network that fully meets the needs of the tissue. The successful manipulation of complex 3D vasculatures necessitates control over microvascular morphology and an understanding of the factors involved in this process. This review focuses on tissue vascularization and its regulation, with an emphasis on the development and control of microvessel morphology and organization.

A. Microvasculatures

The vascular system plays an important role in the maintenance of tissue homeostasis by providing oxygen and nutrients to different organs, promoting the removal of metabolic waste, integrating hormonal communication between distant tissues, and allowing for the rapid response of the immune system to distal sites of the body.[28] Its main component, the blood vessel, is complex and highly structured. The basic structure of the smallest microvessels (capillaries) is an endothelial cell (EC) monolayer organized as a tube. All microvessels have an integral basement membrane that, depending on the microvessel type,[29,30] consists of laminin, collagen IV, and other extracellular matrix (ECM) molecules.[31–33] In addition to forming the core structure of the microvessel, ECs also mediate blood cell activation, fibrinolysis, and coagulation; blood-tissue exchange; and vascular tone.[34] As vessel caliber and complexity increase, perivascular (mural) cells such as smooth muscle cells and pericytes envelop the ECs. Perivascular cells have an important role in vessel stabilization and integrity, and impaired perivascular cell coverage leads to hemorrhagic and hyperdilated vessels, resulting in edema, diabetic retinopathy, and even embryonic lethality.[35]

Establishing an effective microvasculature or maintaining a pre-existing one involves a complex progression of events involving new vessel growth (angiogenesis) coordinated with vessel adaptation to meet the functional needs of the tissue[36] (Fig. 1). In this context, single vessels will grow from existing vessels, persist or regress, change diameter, and/or modulate the mass of the vessel wall based on environmental cues.[37] These processes, often called neovascularization and angioadaptation, involve revising and pruning the network to achieve an efficient perfusion circuit and can depend on the interplay of vascular responses to angiogenic stimuli, the metabolic status of the tissue, and the hemodynamic forces exerted by blood flow.[36–39] Hallmark features of a stable network are morphological and functional heterogeneity among vessels in the network.[40] This heterogeneity reflects a vessel's position within the network (e.g., an arteriole vs. a venule) as well as specific cues unique to the tissue environment. Any successful strategy for constructing a new microcirculation should provide a means by which proper network organization and functional specificity can occur.

B. Microenvironment: Global versus Local

Most tissues contain vascular networks with specific architectures reflecting the varied function of those tissues. For instance, the microvasculature in skeletal muscle is highly ordered, with many of the distal vessels following a specific orientation generally aligned with myocytes.[41] The mechanisms by which vasculatures are directed into a unique architecture are unclear. However, elements intrinsic to the tissue environment, such as growth factor gradients, oxygen gradients, ECM structure, stromal organization, and mechanical force gradients, are most certainly involved. In the broadest sense, there are 2 possible general levels of control of vascular organization: global and local. Global control systems represent a higher-order control that patterns the overall organization of the network within the tissue as well as the overall network boundaries. For example, during embryo development, genetic systems control gene expression gradients across entire tissue beds to organize the tissue and pattern the developing vasculature.[42–45] Studies of tracheal development in *Drosophila* indicate the existence of a genetic control for at least the primordial branching morphogenesis, which in subsequent stages could be modulated by local feedback signals.[46] Similar mechanisms have been envisaged in developing vasculature, where an additional differential expression of genes of the ephrin family has been

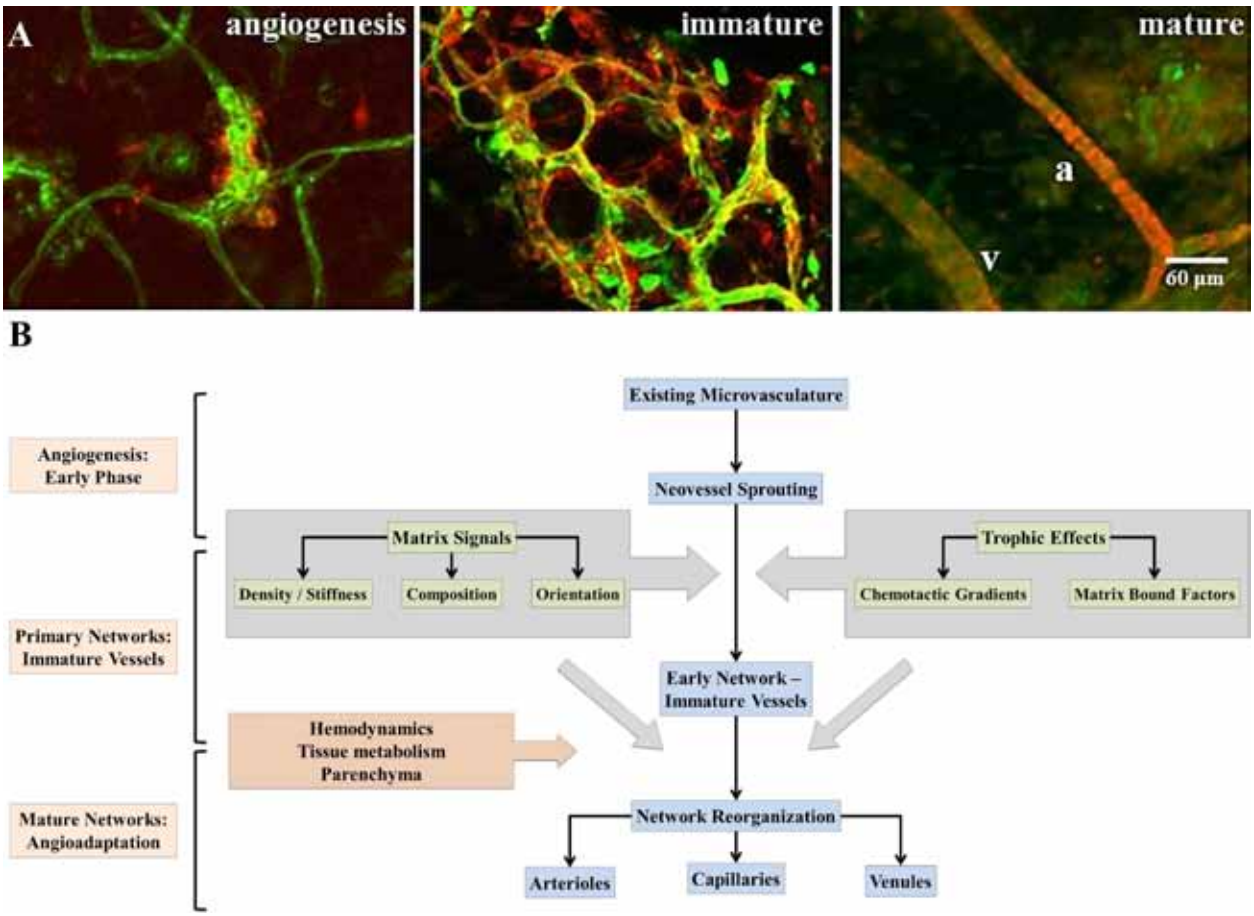

FIGURE 1. Neovascular progression in implanted microvascular constructs. **A:** Progression of implanted microvessels through the stages of angiogenesis and network organization, yielding a new heterogeneous microvascular bed composed of arterioles (a), venules (v), and capillaries (endothelium = green, smooth muscle = red) (Reprinted with permission from Nunes et al.[36]). **B:** Schematic summarizing the integrated input stimuli determining neovascularization outcomes. While similar stimuli affect neovascularization at multiple places in the progression, the vascular responses to these stimuli are likely different

observed in arterial and venous angioblasts even before the advent of blood flow (see Ref. 47 for a review). Whether analogous global systems operate in adult tissues is not known. But genes involved in higher-order regulation in the embryo do play a role in adult angiogenesis.[48] While signals generated at the global level ultimately act on vascular cells, local control reflects signals generated near or at the vessel wall that provide local instruction. The production of angiogenic factors by local stromal or tumor cells is perhaps the best-known example of local control. However, other important local signals include matrix or substrate composition and properties, microstructural changes, cell-cell interactions, and mechanical forces (including fluid flow).[30,49–54] Local control influences more the vascular organization within a network, such as structural adaptation in response to network hemodynamic forces, vessel densities within the network, and vessel specification.[55,56] In general, global regulation acts to organize and pattern local stimuli, which influence elements of individual vessels in the network.[57] Our recent studies have clearly demonstrated that a persistent patterning influence present during normal network maturation and remodeling events, probably induced by hemodynamic alterations, can alter the distribution of its morphological subtypes: arteries, veins, and capillaries.[58–60]

C. Microenvironment: Complexity

In the native tissue, a variety of integrated stimuli act at the local level to give rise to a functional microvasculature. Autocrine and paracrine growth factors/cytokines, in gradients or otherwise, signal to vascular cells in the context of the structural constraints and mechanical influences of an ECM. In addition, vascular cells physically interact with each other and tissue stromal cells to form the vessel structure and navigate the tissue space. The spatial and temporal control of these varied stimuli in the microvascular microenvironment is critical to the dynamics of vascular form and function. For example, ECs exposed to collagen IV, collagen VIII, and laminin of the microvessel basement membrane contribute to the structure of the vessel wall, whereas exposure of the extravascular space to collagen I is thought to trigger angiogenesis.[61] Also, without the recruitment of a perivascular cell layer to the EC tube via orchestrated platelet-derived growth factor–β signaling, microvessels are dilated, fragile, and prone to hemorrhage.[62,63] Conversely, direct heterotypic contact with mural cells and a concomitant dependence of vascular endothelial growth factor (VEGF) has been reported to be essential for formation of human umbilical vein EC networks.[64] Such cell-cell interactions may be necessary to determine the angiogenic phenotype, as highlighted in various sections of this review. Finally, the distribution of mechanical forces through a collagen I matrix and the corresponding organization of collagen I fibrils seem to contribute to the guidance of angiogenic neovessels and overall microvessel position.[65,66]

The formation of a new, stable microvasculature requires the coordinated balance between those stimuli promoting expansion of a vascular bed (i.e., angiogenesis) and those stimuli promoting vascular stability and maturation. While endothelial cell angiogenic processes are stimulated by a variety of growth factors, VEGF has the broadest impact on the vascular endothelium.[67] While not fully demonstrated, the different cell responses to VEGF likely depend on the bioavailability, spatial distribution, and gradient structure of VEGF as well as its coincident signaling with other factors.[64,68–71] As a consequence, because of the potent provascular activity of VEGF, a common strategy to promote vascularization of a tissue construct has been to incorporate it into the 3D matrices of the construct. For instance, matrix-bound VEGF has been used to induce ECs into elongated, sprout-like morphologies in fibrin,[72] and porous poly(lactic-co-glycolic) acid.[73] In addition, environmental modifications such as oxygen tension, which regulates VEGF levels, have been investigated with the fibrin-bead system.[74] Concomitant with responses to growth factors, an EC must respond to the mechanical loading caused by other cells either through direct cell-cell contact or indirect matrix remodeling.[75] The contact inhibition of EC-EC interaction is a dominant regulating influence, maintaining endothelial quiescence.[76] Also, EC shape may influence proliferation, and even a linear increase in the area over which cells spread can cause an exponential increase in the DNA synthesis, unlike simple receptor occupancy without any cell extension.[77] Since EC shape is maintained by a balance between the tension exerted on the focal adhesion sites and the resistance offered by the substrates (tensional homeostasis), it stands to reason that a mechanochemical coupling exists, resulting in additive effects or increased responsiveness.[78,79] Furthermore, cells impart strains onto matrices to facilitate cell growth,[80] and cell alignment is associated with static and dynamic growth within stressed and unstressed matrices.[32,81,82] In a similar way, the degradation and focal proteolysis of microvessel basement membranes is hypothesized to change the ECM compliance, thereby altering the tensional homeostasis of the ECs with their neighbors, initiating sprout formation inherent in angiogenesis.[83] From these limited examples, it is clear that the cellular microenvironment consisting of other cells, growth factors, matrix structure and composition, and the mechanical milieu are critical in determining vascular cell phenotype and function.

II. MICROVASCULAR ASSEMBLY

A common goal for the assembly of vasculatures involves *in vitro* blood vessel fabrication of both small- and large-caliber vessels. In addition, such constructed vessels and vasculatures can be used to replace both damaged vascular beds and dysfunctional ar-

teries and veins and further our understanding of the mechanisms directing vascular dynamics and remodeling. There are 2 general approaches in microvessel formation: vasculogenesis (the formation of vessel structures by the coalescing of cells)[13] and angiogenesis (the formation of new vessels from preexisting blood vessels).[31] Two-dimensional, cultured, cell-based systems generate the cell sources needed and provide a means to investigate relevant responses of cultured microvascular cells (e.g., ECs, smooth muscle cells, and pericytes) to growth factors,[55,84–86] other signals produced by cells,[87,88] cell-cell interactions,[64,89,90] and combined growth factor–matrix signals.[91–93] For example, confluent layers of cultured ECs spontaneously form tube-like structures, which invade the underlying matrix and enclose a lumen (vacuolar or otherwise) to form structures resembling capillary sprouts.[30,33,94] While these and similar approaches provide many of the mainstays of the investigative approach into components of the angiogenic process (and microvascular assembly), many of the models suffer from the lack of a physiologically relevant physical environment. However, with the realization that cell phenotypes in isolated cell systems differ between 2-dimensional substrates and 3D scaffolds,[61] many models have incorporated a 3D variation of this tubulogenesis or capillary-like structure–forming approach. In addition, other models involve the use of vascular structures, which comprise the necessary vascular cells already in a native vessel structure.[31,95–97] In all cases, maintaining cell viability and functionality can be difficult, in particular when using human cells. Modification of the vascularizing cells often is required, either through genetic modification to increase resistance to cell death[98] or in combination with circulating progenitor cells, to improve the viability and behavior of current and future engineered cells.[99]

A. Isolated Cell Systems

The majority of engineered microvessel systems, both *in vitro* and *in vivo*, use isolated (and usually cultured) vascular or prevascular cells as the basis for forming new microvessels. Here, vascular precursors are induced to assemble vessel-like structures that can be capable of progressing into mature microvasculatures. The use of isolated cells in vascularizing tissue constructs makes it possible to populate a wide variety of scaffold or matrix systems as well as use flexibility in construct design. The cultured cells typically are placed in an environment that promotes assembly into "capillary-like" structures.[100] A common approach to forming neovessels is to culture vascular cells on polymer beads embedded within an facilitating ECM such as fibrin,[101,102] collagen,[103] or Matrigel.[104] In these systems, EC chains grow out from the bead-culture. In addition to providing a strategy for assembling new vessels in regenerative applications, such *in vitro* systems have proven useful as simple angiogenesis assays. A majority of the cells used in this generic approach are vascular cells such as ECs,[105] vascular smooth muscle cells,[106,107] and pericytes,[84,89,108] with some also using stem cells.[109,110] However, applications using only a single type of vascular cell may lack appropriate cellular feedback between the different cells thought necessary to assemble microvessels,[30] prompting the incorporation of perivascular cell precursors with the ECs.[111] Such combinations of cells have been used, for example, in the engineering of vascularized skeletal muscle tissue to maintain viability, with about half of the total number of vessel-like structures perfused at 2 weeks.[112]

B. Isolated Vessel Systems

Different from isolated cell systems, isolated vessel systems use "organ culture" of elements native to the vessel. Suspension of the aortic ring or inferior vena cava segments in a 3D matrix such as collagen is a common strategy in angiogenesis models.[95–97] With this technique, ECs extend from the edges of the excised large vessels, forming capillary-like morphologies that project into the surrounding matrix. As the EC cords develop, the aortic ring assay provides insight into cell recruitment, matrix degradation, cell proliferation, and cell migration.[113] Such assays have been used to evaluate tumor growth and the effects of angiogenic and antiangiogenic treatments.[114] The culture or reimplantation of *in vivo* prevascularized constructs is a variation of such assays. When implanted, ECs extend from such constructs to form cord-like networks and have been shown to inoscu-

late with each other and the host circulatory system.[115] In another approach, isolated microvessels—instead of macrovessels—are collected and seeded into collagen I gels.[31] The characteristic feature of this approach is the spontaneous, *in vivo*–like sprouting angiogenesis that occurs from the intact microvessels, which serve as parent vessels for the angiogenic sprouts. Once implanted, the nascent neovessels spontaneously connect to the host circulation and remodel to form a perfused microcirculation with proper in-flow and out-flow pathways.[19,36,116] The progression of these implanted neovessels, whether derived from parent microvessels or other precursor sources, into a perfused, hierarchical network highlights the intended outcome of any vascularizing strategy (Fig. 2). Regardless of the source (aorta or microvessel or isolated cells), successful strategies must consider dynamics after angiogenesis to derive a new, functional microvasculature.[117,118]

C. Bioreactors for *In Vitro* Vascularization

The onset of flow is a key mediator of microvessel development *in vivo*.[19,119,120] Therefore, to replicate such development, a method to expose constructs to flow first and then maintain those conditions to propagate network development is required. Simpler experiments circulate media across cell

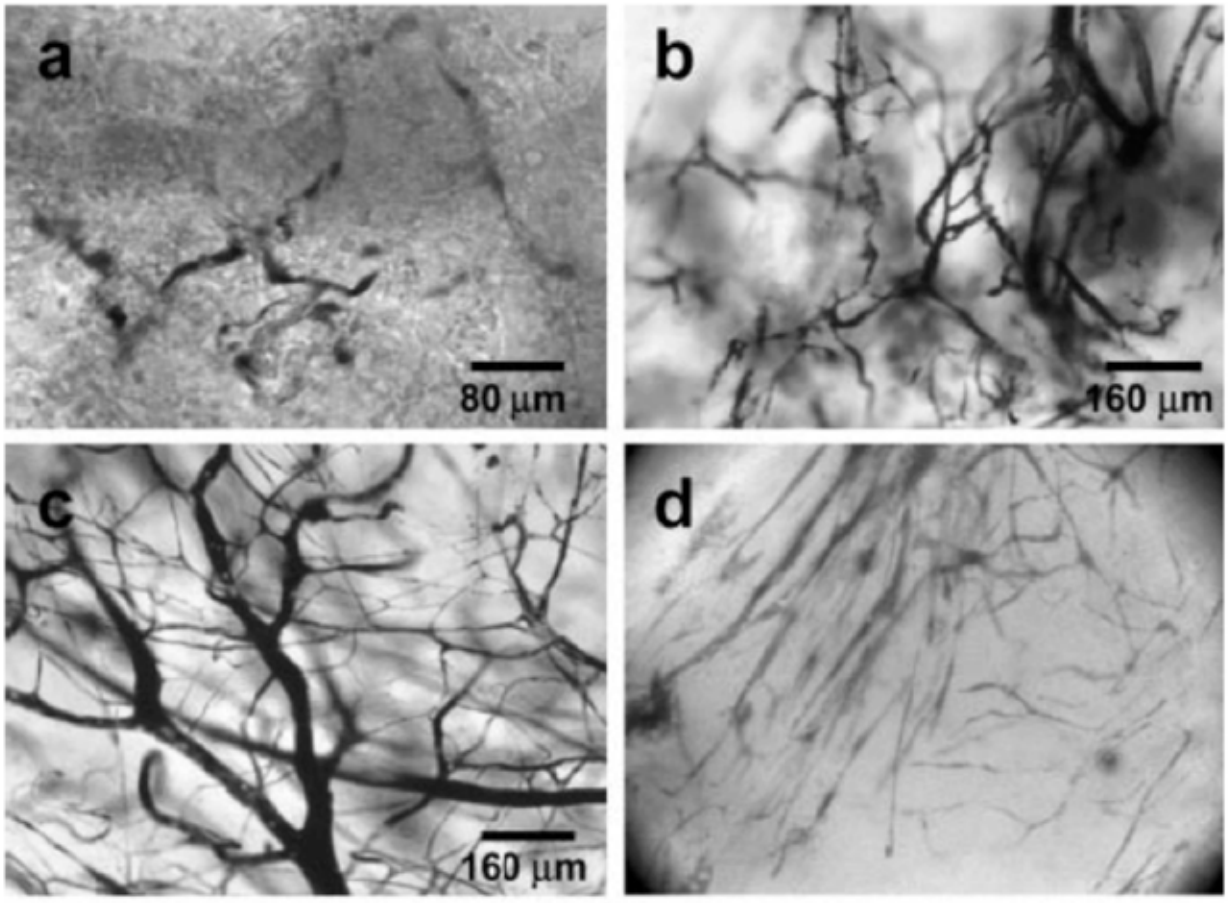

FIGURE 2. Progression of maturation after angiogenesis in an implanted neovasculature. Vascular casts of progressively longer neovessel implants made from host animals perfused with India ink: Soon after implantation (day 1) (**a**), limited neovascular network segments are perfused via the host circulation. Days later (day 2, **b**; day 3, **c**) nearly all of the immature network is perfused and eventually remodels into a more hierarchical microcirculation by day 28 (**d**). (Reprinted with permission from Shepherd et al.[19]).

monolayers[121–123] or thin films of cells suspended in a 3D matrix to create maturing shear forces.[124–129] Although capillary-like structures have been identified within these systems, functional networks of heterogeneous diameters are yet to be realized. In more complex biochambers, cells are suspended in media and circulated within a sterile container[130] or held within hollowed 3D scaffolds sodded with cells.[131,132] Hollow fiber bioreactors also have been used to assess cellular function in the presence of flow.[133,134] While these systems provide insight into cellular behavior in these more complex conditions, they are less conducive to vessel fabrication *in vitro*. Still more complex bioreactors incorporate perfused, harvested vessels or vessel constructs; engineered blood vessels mimics such as decellularized cadaver vessels,[135] porcine arteries,[136] and human saphenous veins[137]; peripheral blood–derived blood vessel mimics[138,139]; and synthetic cylindrical polymer scaffolds[140] to generate vessels as small as 4 mm in diameter.

Although promising for large-vessel applications, 4 mm is still orders of magnitude larger than typical arterioles and venules found in the microvasculature. To reduce the size of experimental *in vitro* vessels, smaller-caliber channels have been formed by drilling cylinders into collagen sponges[117] or polymerizing collagen around mandrels of varied caliber.[118,141] After sodding the structures with microvascular cells, the constructs contained vessel-like structures with internal diameters a small as 150 μm. Similar results have been obtained by directly sodding cells onto nylon tubing.[23] In all of these models, sodded cells formed endothelial monolayers on the luminal surface of the perfusion conduits. In many cases, ECs and mural cells migrated out into the surrounding matrix, forming networks of cord-like structures adjacent to the internal lumen.[23,105,118,141] While endothelial sprouts are capable of invading the surrounding matrix,[117,118,142] mimicking angiogenic behavior,[143] evidence of network patency and function has not been definitively provided. Therefore, true vascular network development *in vitro* has yet to be realized.

Toward this goal, bioreactor designs that incorporate a means to provide intravascular perfusion of *in vitro* neovessels are being explored. An interesting challenge is the drop in scale, often in diameter, in the flow paths needed to perfuse the narrow neovessel segments. Assuming the neovessels are interconnected, appropriately stepping down from the external pump tubing (millimeters in diameter) to neovessels that are less than 10–15 μm in diameter is difficult. One approach has been to use micro-flow systems, such as those in microelectromechanical systems platforms, to scale down the entire perfusion circuit to the microvascular scale.[144,145] While very useful in a variety of applications, these "chip-based" microvascular systems are difficult to integrate into a vascularized implant. In a different approach designed to accommodate larger tissue volumes (e.g., >3 mL), a macroscale bioreactor designed to incorporate a contrived conduit or "feed vessel" into the 3D tissue construct can be used. Under the appropriate conditions, growing neovessels could inosculate with this feed conduit and, assuming lumen continuity, become perfused.[143] It is interesting that angiogenesis in this larger system occurred only under what would seem to be suboptimal conditions for oxygen and nutrient delivery,[143] highlighting the complexities of forming vascularized tissue systems or mimics *in vitro*.

D. Prevascularization of Implant Sites and Accelerated Biomaterial Vascularization

An alternate strategy to prevascularizing tissue constructs involves implanting avascular constructs into prevascularized tissue sites. This commonly involves the use of implanted growth factor depots to stimulate host angiogenesis at the implant site that subsequently progresses into the construct. For example, incorporation of fibroblast growth factor-2 within 3D matrices, including heparin and alginate,[146] silk fibroin,[147] collagen,[148] and fibrin,[131] has been used to increase neovessel formation and host cell infiltration in a variety of applications such as bone implants,[149] cultured skin,[150] and hernia patches.[151] VEGF has been incorporated into similar materials[119,152] and bioresorbable polymers to slowly release it into the surrounding tissues.[73] Delayed or controlled release of growth factors can increase the infiltration of host vessels into implanted tissue constructs.[153,154] An alternate approach relies on the targeted placement of

vascularizing cells that form vessel structures and/or release angiogenic factors at the implant site preceding construct implantation.[73,155] For instance, a porous poly(ethylene glycol), poly(lysine), and poly(lactic-co-glycolic) acid scaffold was sodded with neural progenitor cells and ECs and then implanted into severely compromised immunodeficient mice.[156] After 6 weeks, high densities of tube-like structures were found within the matrix. Similar results were attained by implanting a Matrigel-based EC and 10T1/2 cell construct into severely compromised immunodeficient mice.[119] After 4 weeks, neovessels formed within the matrix and were perfused by the host circulatory system. In another example, an implant consisting of Vicryl mesh (90:10 poly[glycolide:lactide]) infiltrated with human fibroblasts secreted a number of growth factors into the surrounding tissue, thereby increasing microvessel density 2-fold.[157] An approach involving the preimplantation of cells can also take advantage of genetic modification strategies to customize factor production at the implant site.[158] Finally, implant sites are being prevascularized through the surgical manipulation of existing vascular beds. In one technique, a graft between the femoral artery and femoral vein of a rat created an arteriovenous loop within a semisealed chamber, in which a functional microvasculature expanded over time.[159] Tissue specific microvessel beds can similarly be excised as whole tissues and processed for subsequent reimplantation.[160–162]

E. Microvessel Derivation from Tissue Explants

A variety of strategies for deriving new microvessels in culture from explanted tissues can be used. In these approaches, all necessary vascular precursors (e.g., ECs, murals) are included in the tissue harvest, facilitating the formation of new vessels. For example, in cultured murine myocardial tissue harvests, new interconnected vessel segments arose following 3 weeks of culture.[162] In other approaches, the tissue explant is used to establish the stromal architecture supporting microvascular networks. After decellularizing harvested segments of porcine bowels, repopulating the tissue scaffold with endothelial progenitor cells "re-derived" the microvascular network.[161] In a similar way, placental tissue has been excised, decellularized, and resodded to form *in vitro* networks.[160] Others have harvested retinal microvasculature and directly perfused them to study microvessel behavior *in vitro*.[163] These procedures each provide *ex vivo* experimental microvasculatures and serve as useful platforms for network investigation and as biologically derived scaffolds for tissue generation, yet these techniques lack the ability to easily modify construct dimension and scale. The systems also require lengthy procedures to harvest tissue and weeks of sample conditioning before the networks are ready for experimental use. While these models retain patent microvasculatures for *in vitro* perfusion, the networks are organ and tissue specific, with vessel concentration and topology optimized for the original tissue, and offer limited opportunity to direct neovessel growth. Therefore, these systems may not be conducive to the development of varied tissue types. They may also limit potential construct designs by requiring future engineered tissues to be designed around the available vessel topology of the excised network. There may be no ideal *in vitro* angiogenesis system that is a perfect physiological mimic,[164] but the very recognition of this fact helps in the careful interpretation of results from the various prevalent models.

III. EXTRACELLULAR MATRIX

The ECM is a significant determinant of microvessel form and function. Every aspect of the ECM, including its composition, density, stiffness, fiber orientation and spacing, perceived porosity, and tensional state, all have a regulatory influence on vascular cells. Either as a simple biochemical signal or a spatially structured milieu of matrix-bound factors, the matrix regulates a variety of vascular cellular activities such as migration, proliferation, polarity, maturation, and phenotypic expression. However, the relationship between ECM ligand signaling and mechanical coupling in vascular biology remains incompletely described. It is clear that ECM ligand and cell-receptor binding alone are insufficient to determine microvascular pattern and growth. For a given substrate, cells may be directed to proliferate or differentiate depending on the mechanical attributes.[94] Hence, both the ECM structure and its me-

chanical properties are relevant to development of a 3D vasculature. Regardless, given that initiation, elongation, and guidance of the angiogenic sprout or vasculogenic tube critically involve the spectrum of cellular activities,[165–167] any strategy to manipulate a functional microcirculation via microvessel assembly must consider the ECM.

A. ECM Composition

The composition of the ECM can modulate EC activities (proliferation, monolayer confluence, basement membrane formation, and tube-like structure formation).[33] ECs from adipose tissue tufts cultured on basement membrane collagens (IV, V) aggregated and progressed to form branched, tube-like structures, whereas those on interstitial collagens (I, III) simply proliferated and spread to confluence. ECs showed a similar response on the basement membrane and stromal side of amniotic membranes.[33] Montanez et al.[168] found differences in lumen morphogenesis between Matrigel- and collagen I–based angiogenesis models, which may be related to the difference in stiffness between these 2 substrates, among other factors.[169] Collagen as a substrate for growing vascular cells was pioneered by Ehrmann and Gey[170] in 1956 and, along with fibrin, has since become one of the ubiquitous substrates in the study of ECs and network formation. In addition to collagen I and basement membrane proteins (collagen IV and laminins), other relevant ECM molecules include collagen VIII, a promigratory molecule with low celladhesiveness[171] that is associated with rapidly proliferating cells[172]; the proteoglycan Decorin, which is causally involved in formation of EC cords with lumen (sprouts)[173–175]; and Tenascin C, which is cytoprotective and promotes EC migration, cord lengthening and branching, and network formation.[176,177]

B. Vascular Cell–ECM Coupling

While not the only mechanism involved, the integrin-cytoskeleton axis is the primary molecular system coupling cells to the ECM. Integrins are multidomain transmembrane proteins serving a variety of roles in the mediation of cell-ECM interactions.[83,178] Integrins serve as the interface between the intracellular structural framework of the cell, composed of actin filaments and microtubules (i.e., cytoskeleton), and the 3D structural framework of the tissue (i.e., the ECM). It is this interfacial location that implicates integrins in mechanotransduction by not only transmitting external forces to the cell's structural elements but also via intracellular second messenger systems activated by these perturbations via their binding to specific ligand moieties on the ECM. The integrins are central regulators of vascular cells; deletions of the β_1 subunit lead to embryonic lethality due to vascular defects.[179] ECs treated with anti-integrin antibodies (α-chain specific) reduced proliferation and increased tube formation.[166] Integrin subunit involvement varies with the type of substrate and cellular activity. The α_2 and α_5 subunits are the predominant collagen- and fibronectin-specific receptor complex components.[180] During angiogenesis, the integrin expression profile of ECs changes from $\alpha_1\beta_1$ to $\alpha v\beta_3$ (or $\alpha v\beta_5$), which is dramatically upregulated compared to their near absence on quiescent ECs.[178,181–183] Furthermore, in response to VEGF, integrins $\alpha_1\beta_1$ and $\alpha_2\beta_1$ are upregulated, thus showing a modulation of cell surface adhesivity.[178,181–183] Finally, Malan et al.[179] demonstrated that the lack of a β_1 integrin subunit can prevent the deposition of ECM elements such as collagen IV, laminin, and fibronectin in a pattern that either is supportive or indicative of vascular tube-like structures. This reveals a critical feedback between integrins and ECM ligands.

C. ECM Structure and Stiffness

Numerous examples demonstrate that ECM stiffness influences cell behaviors. In general, cells migrate more slowly and spread better on stiffer matrices,[184–186] whereas proliferation is reduced on softer matrices.[184] Fibroblasts cultured on flexible substrates of similar biochemical composition showed increased motility or lamellipodia ruffling but were less spread, as on stiffer substrates.[187] Furthermore, cellular phenotype can depend upon the type of ECM substrate and its stiffness.[188–190] However, phenotypic modulation can depend upon the cell type and other conditions such as cell density. For example, IMR90 fibroblasts (an immortalized fibroblast line) form cords and contract collagen

gels, but only in stiffer matrices, whereas primary fibroblasts do so in a matrix of lesser stiffness.[54] Lo et al.[186] observed that such stiffness-dependent cell phenomena were observed only at lower cell-seeding densities, pointing to a more complex situation involving cell-cell interaction and subtle changes in local substrate mechanics. It is interesting that matrix microstructure is thought to determine, in part, the amount of strain transferred to the cells, thus relating matrix structure and stiffness to the regulation of cell function.[191] Cell migration involves directed cytoskeletal contractions and tension that are a function of substrate stiffness.[192,193] Related to this, cell stiffness, presumably due to a concomitant increase in the organization of actin fibers, increases with increased substrate stiffness.[184] Also, integrin-cytoskeletal linkages are strengthened in response to perceived changes in substrate rigidity.[187,194] This linkage strength is dynamic and a function of integrin organization. Such dynamic alterations in focal adhesion complexes support the idea that these complexes are important sensors and regulators of the mechanotransduction process.[187,189,194–196]

The ability of a substrate of defined stiffness to sustain multicellular retraction can also affect the formation of capillary-like structures (CLSs).94 While relatively low matrix stiffness resulted in rounded cells with poor viability, high matrix stiffness supported normal cell viability but prevented the formation of CLSs, suggesting that an ideal range of matrix stiffness is important for cell aggregation. Both the matrix density and cellular mechanical environment influences the formation and structure of CLSs, reinforcing both the role of the integrin-cytoskeleton axis as well as the possible existence of a balance in the cell forces and matrix stiffness.[94,182,197,198] In a more complex model of neovessel sprouting, we demonstrated that the normalized dynamic stiffness of vascularized collagen I constructs decreases sharply during sprouting and early network formation but returns to higher values during neovessel elongation and further network growth.[199] It is interesting that the total normalized proteolytic activity in the construct correlated inversely with changes in construct stiffness but the expression of matrix metalloproteases remained elevated.[199] While the changes in matrix stiffness did not seem to affect the integrity of the neovessels formed (which does not involve a vasculogenic process), they did correlate with changes in neovessel activity (sprout formation vs. neovessel elongation and network formation). The potential involvement of matrilysis in changing matrix stiffness, however, agrees well with earlier studies of tubulogenesis or resorption.[200,201] It should be noted that the ECs of the microvascular construct model interact with the interstitial collagen of the surrounding gel as intact, growing neovessels and not as individual cells.[31] Changes in matrix density and stiffness can also affect EC structure morphology and network and topology. For example, pulmonary microvascular ECs cultured on collagen matrices of different stiffness show remarkable differences in invasiveness and the topology of CLS networks due to pH-dependent polymerization changes.[169] More flexible (i.e., less stiff) matrices promoted the formation of thinner superficial networks with "vacuolar" lumens, whereas stiffer matrices support thicker and deeper networks with true cell-lined lumens.[169] In a similar way, stiffer matrices of fibrin with higher ECM protein concentrations produce less extensive capillary-like networks.[102,202] The dependence of ECs on the matrix structure for 3D assembly is further reflected in the markedly slower rates of tube formation in 2-dimensional (days) versus 3D (hours) matrices (reviewed in Ref. 166).

How matrix stiffness influences vascular cell behavior remains unclear. Evidence from the current literature suggests the existence of a dynamic mechanical balance between the cell and the surrounding ECM. However, Ghajar et al.[202] proposed that differences in hydrogel rigidity can influence diffusion kinetics, which in turn can affect network growth. It is also possible that changes in the stiffness or density of a matrix may alter proteolytic activity.[203] Care should be taken when using proteolytic inhibitors to address this since inhibition of matrix metalloproteinases (MMPs) can themselves lead to direct inhibition of angiogenesis.[204,205] Porosity of the matrix environment, a function of matrix density, could also be involved. However, it is likely that porosity plays a permissive role such that the pores are either too small to permit cell invasion or large enough to accommodate a cell (or microves-

sel) presence. Vascular expanded polytetrafluoroethylene grafts with an intermodal distance of 60 μm readily permitted the microvasculature to grow into the graft wall.[206–208] A membrane with porosities ≤0.22 μm clearly does not permit cell invasion.[209]

D. ECM Remodeling

While the matrix environment influences vascular cells, the activities of vascular cells also influence the matrix, which can in turn reinfluence the cells. Matrilysis during angiogenesis, particularly by MMPs, is a well-described phenomenon[210] and likely represents the best known example of matrix remodeling. Recent evidence from a 3D model of angiogenesis correlated the changes in mechanical properties of the tissue environment with total proteolytic activity during angiogenesis, suggesting that matrilysis of the bulk matrix may be important as well.[199] In addition, new matrix production and the deposition of collagens, fibronectin, laminins, glycosaminoglycans, and others occur throughout angiogenesis and neovessel formation (see Ref. 178). A third means by which vascular cells remodel matrix is by restructuring matrix features and components. For example, the spontaneous formation of CLSs from cell monolayers involves the generation of condensed collagen fibrils called adhesive tendrils,[94] traction fibers,[211,212] or matrix migration pathways,[32,211,212] which are thought to direct cells to migrate and proliferate to form tube-like structures.[82] Stopak and Harris[213] reported the development of linear tracts of collagen fibers as long as 4 cm by contracting fibroblasts, which were more pronounced in stressed matrices. These changes in collagen structure were due to local contraction of the fibroblasts,[213] which can happen as soon as 30 minutes after seeding.[214] The changes in the lattice structure of collagen fibers in these examples is likely due to simple reorganization of existing collagen since there was minimal new collagen turnover associated with this large structural change.[214] During angiogenesis in a 3D collagen matrix, collagen fibers are bundled along the wall of the growing neovessel and oriented along the axis of the neovessel ahead of the advancing tip.[65] While not examined in this study, presumably the restructuring of the collagen lattice was due to vascular cell contractions related to the elongating neovessel, similar to that described for fibroblasts. ECs and fibroblasts actively contract collagen gels and artificial scaffolds.[81,215] Interestingly, contraction of collagen matrices by ECs is inversely proportional to the starting matrix stiffness.[102]

An interesting point to consider is the integration of simultaneous matrilysis and the generation of tractional force that is depending on cell attachment. The matrix protease MT1-MMP and β_1 integrin colocalize on the forward leading edge of cell processes, followed by a trailing proteolytic zone responsible for the actual matrix remodeling.[216] Transient clusters of MT1-MMP at the leading edge of the cell do not cleave fibrillar collagen (thought to be due to stearic hindrance) and serve as adhesive anchors, whereas similar coclustering of integrin and MT1-MMP leads to active matrilysis.[216] A similar regulation of MMP-2 activity via a non-RGD (Arginine – Glycine – Aspartic Acid)-dependent binding to integrin $\alpha v\beta_3$ could also be involved in focal matrilysis.[217] The idea that adhesion and matrix remodeling are coupled is reinforced by the observation that subconfluent and migratory cells generated higher traction than confluent or quiescent cells and that the effect was diminished by MMP inhibition.[215] This might explain how ECs within a growing angiogenic neovessel can simultaneously exert traction and process ECM. Coordinately, the active process of pulling or compressing matrix molecules as a result of cell-dependent tensional forces may affect matrilysis. Stretching the ECM can alter protease binding and/or cleavage sites of individual matrix molecules, thereby changing the ability of proteases to degrade the molecules.[218]

E. Contact Guidance

Since its first description, contact guidance has been recognized as a determinant of cell locomotion and orientation. *In vivo*, the oriented ECM fibers and 3D organization of the stromal tissue provide cells with a well-defined substrate for growth and form the basis for contact guidance. Spatially controlled cell adhesion on surfaces is one of the prevalent forms of cell patterning.[219] Printed guidance channels of ligands such as fibronectin,[220,221] surface-

etched features like ridges and grooves,[195,222–224] or engineered fibers[225,226] have formed the mainstay of these orientation strategies. However, while cells in general prefer to grow along such adhesion or contact guidance paths, there seems to be a differential sensitivity to guidance features such as width, groove dimensions, pore morphology, and ligand density.[219] Central nervous system neuroblasts and peripheral nervous system neurons cultured on patterned surfaces mimicking neurite bundles showed orientation in both directions with a dependence on width and depth.[227] Broadly speaking, substrates with ridges or grooves narrower than the diameter of the cell produce maximal alignment of cells along the substrate features.[195] Alignment of cells along grooved substrates increases with increasing feature depth (200 m to 5 μm)[195] but reduces with decreasing groove width.[222] However, the migration velocity remains unaffected, possibly as a function of the typical sizes of the focal adhesion complexes, and their distribution relative to the ridges and troughs.[222] It is interesting to note that the percentage of aligned cells on all grooved surfaces changes during longer culture periods.[195] Narrow adhesive patterns (about 20 μm wide) were able to constrain migration and orient growing ECs along their long axis better than relatively wider tracks, similar to that seen in ridges or groove-like patterns at confluence.[228] Such cells were maximally elongated on 24-μm track widths with a range of adhesion widths.[229] Although it is difficult to gauge the height of these patterns, it is clear from other studies that even a depth of a few hundred nanometers up to 1 μm is sufficient to generate a patterned migration.[230] Still, it is interesting to note that even at the widest adhesive channel (130 μm), the cells show preferential orientation of their long axis along the pattern's long axis at confluence.[228]

Guided contact of cells involves a balance between intracellular forces, cell adhesion, and the mechanical microenvironment. Harris et al.[231] demonstrated that tractional forces of cells vary with cell type; the more invasive and mobile types generate the least traction. In a similar way, EC motility has a biphasic dependence on cell adhesion such that there is an optimal, intermediate adhesion strength that gives rise to maximal migration.[232,233] Cell-cell interactions in higher-density cultures can modulate cell response to substrate stiffness via formation of adherens junctions[234] and likely influence cell stiffness.[235] These relationships reflect the dynamic equilibrium between adhesion density and cellular tension. Ingber[79] and Chicurel et al.[236] proposed a tensegrity model to explain this equilibrium, whereby forces transduced across integrin adhesion sites are simultaneously distributed throughout the cell cytoskeleton, resulting in adjustments in molecular activity. Contractile cells such as fibroblasts typically generate forces larger than those required to maintain locomotion.[231] Perhaps as a result, matrix fibers around the cells become differentially oriented, with fibers at the ends of elongated cells being aligned with the long axis and fibers at the center of the cells being aligned perpendicular to the long axis. Whether the fiber alignment simply reflects a change in matrix structure as part of a force-equilibrating process or serves as contact guidance tracks (or both) is not clear. Aligned collagen fibers, either by fresh deposition by aligned cells or via force-dependent remodeling, facilitate cell-ECM interactions and contact guidance.[224] Whether such contact guidance phenomena influence vessel formation and angiogenesis is unknown. In a monolayer invasion assay, endothelial sprouts grew along collagen fibrils,[237] suggesting a role for contact guidance in directing cell invasion. However, the cells also moved into interstitial spaces beyond the apparent intersections of collagen fibrils,[237] suggesting that contact guidance is not necessary. In strained, 3D angiogenic cultures, neovessel growth was parallel to aligned collagen fibrils,[66] but because this occurred in an oriented strain field,[238] it is yet unclear if neovessel orientation was due to contact guidance (via the aligned collagen fibrils) or the strain field.

IV. MECHANICAL LOADING

During angiogenesis, EC sprouts and growing neovessels are potentially exposed to both luminal and abluminal mechanical loading. In patent neovessels, luminal forces include fluid shear and circumferential stretch secondary to hemodynamic forces. Abluminal forces arise from vascular cell coupling to the ECM and/or interstitial fluid shear.

In the assembly of any microvasculature, these forces and their consequences on the vasculature should certainly be considered. However, the manner in which forces are applied matters to the outcome. For example, shear-induced angiogenesis primarily progresses by intussusception, whereas the stretch-induced or exercise-induced angiogenesis is of the sprouting type with its dependence on proteolysis by MMPs for basement membrane degradation.[61,239–241] We have evidence that blood flow into neovessels is required for the successful progression from an immature network to a mature, hierarchical network.[36] Also, hemodynamic forces are critical in determining the caliber and wall morphology of individual microvessel segments within an existing mature network.[242] Finally, concomitant application of both circumferential hoop strain and shear stress has a synergistic and potentiating effect on EC alignment.[243] Considerably less is known concerning the effects of interstitial shear and environmental mechanical loading on establishing microvascular form and function, but constraining the deformability of the matrix environment around a forming microvasculature has a profound influence on the topology of the final microvascular network.[60]

A. Tensional Forces

It is well known that static or cyclic stretch can induce cellular orientation either in the direction of or orthogonal to stretch direction.[244–249] Constraining the ability of cell-seeded constructs to contract in a specified direction gives rise to both cell and fiber orientation in a preferred direction.[213,214,250–252] Shirinsky et al.[245] demonstrated a resistance to deformation by ECs (with elongated morphology oriented orthogonal to strain direction) pregrown to confluence under intermittent strain, whereas monolayers established under stationary conditions showed zones of denudation when subjected to stretch. Many cell types, such as fibroblasts smooth muscle cells, and ECs, orient transverse to the direction of cyclic stretch coincident with the formation of similarly oriented actin fiber bundles.[247,253–258] Under stressed conditions, reorganization of the actin cytoskeleton precedes cell orientation[245,259] and depends on the reorganization of integrin-based focal adhesions along the axis of stretch.[180,260] The orientation angle relative to the stretch direction depends on the magnitude of stretch rather than strain rate, at least in the early stages of the perturbation.[247,253,256–258,261] In more complex stress fields, cells and fibers orient circumferentially when allowed to contract axially against radial anchors.[251] In cell-containing matrices assembled around but not adhered to mandrels, cells and fibers also aligned circumferentially; when the matrix was adhered to the mandrel they aligned radially.[250,252] Cells do respond to compression forces[258] but show no specific orientation under equibiaxial planar stretching,[247] suggesting that the stimulus for orientation is cell strain (i.e., a change in cell length) and not tension.

It is not clear why cells orient with or against different strain regimens. One of the prevalent hypotheses is that the cells align in an attempt to minimize exposure to large deformations. Eastwood et al.[262] suggested that cells orient in the direction of maximum principal strain to minimize exposure to the strain field, especially when adopting a long and thin morphology. Others have proposed a mechanism of stretch avoidance reaction in an attempt to minimize the strain acting on the cells and limit peak-to-peak axial deformation.[253–255] However, such a mechanism would involve thresholds because small strains (e.g., 2% stretch) did not induce a specific orientation.[253,256] Neidlinger-Wilke et al.[261] noted that the cells typically oriented perpendicular to the stretch direction, such that the resulting axial strains were less than 5–6%, indicating both a level of threshold strain and a window of strain magnitude for an orientation response. Takemasa et al.[257,263] argue that the oblique alignment of the stress fibers in a cyclically strained substrate acts to minimize the amplitude and dampen the oscillations of axial strain felt by the cells. The direction of strain, the strength of the cell attachment, the nature of substrate, 2D versus 3D strain fields, and other boundary conditions clearly contribute the a cell's response to tensional forces. Unlike individual cells, cellular structures such as microvessels will likely respond differently to applied stress and strain fields. Not all cells in the structure will experience

the same degree and character of force, and the large, relatively bulky elements will be less free to reorient. Thus, neovessels growing within a 3D construct experiencing constant isometric stress or strain along a single axis surprisingly aligned parallel to this axis.[66] They also do this in the presence of cyclic stretch,[66] unlike single cells, which reorient perpendicular to the direction of cyclic stretch.

B. Mechanical Equilibria

In any tissue, cells are in mechanical equilibrium with their microenvironment.[196] Disturbances in this equilibrium can lead to changes in cell phenotype and tissue character. For example, chronic unloading of tendons leads to an increase in protease production, contributing to tendon fragility. Low-magnitude, low-frequency cyclic loading of tendons removed from the body prevents this protease production.[264–266] In blood vessels, vascular smooth muscle cells, which undergo regular, periodic stretch, will experience hypertrophy and produce matrix following chronic increases in stretch (as in hypertension) (reviewed in Ref. 267). In assays involving the assembly of tube- or cord-like structures, the cells do not go on to form structures without a minimum level of mechanical loading because of the matrix's resistance to cell contraction.[268] In one example, a minimum force, corresponding to about 20% of the endogenous forces generated by the cells themselves, was required to elicit a cellular mechanical response.[269] Matrix fibril reorientation and restructuring may represent "mechanical slippage," causing cells to migrate toward stiffer areas of the matrix.[185,186,270,271] The establishment of mechanical equilibrium suggests that cells have an ability to sense balanced tensional forces. This is likely intrinsic to the complex and integrated system of adhesion complexes, cytoskeleton, and cellular contractile machinery,[82,186,213,250,272] which will continually adapt and organize until a mechanical homeostasis is established.[273–275] This may explain why even though fibroblasts orient monotonically with increasing alignment of collagen fibrils, there seems to be a threshold level of mechanical loading that is necessary even in highly oriented fiber matrices.[276] Conversely, the relative magnitude of cell traction and adhesion dictates more capillary-like morphology than absolute substrate stiffness.[198]

C. Mechanical Forces versus Contact Guidance in Angiogenesis

In nearly all cases, the architecture of the microvascular microenvironment is coupled to mechanical loading. Anisotropic matrices will transduce forces differently than isotropic matrices. Mechanical loading of a tissue or the presence of cells within ECM scaffolds can lead to changes in matrix structure and fibril organization (Fig. 3). Therefore, which of these general stimuli, mechanical loading or contact guidance (via matrix organization), is the dominant influencing factor on tissue and vascular cells is not always clear. As we manipulate the tissue microenvironment to assemble a neovasculature or alter an existing microvascular bed, it is important to understand the contributions of each to the process. While it is difficult to uncouple loading effects from contact guidance, it seems that in general contact guidance strongly influences vascular cell organization. Adhesion-dependent cells certainly require a substrate upon which to attach. As a consequence, it is reasonable to assume that the organization of these substrates will affect the organization of the attached cells regardless of the loading conditions. For example, in a 3D collagen gel containing aligned fibronectin strands experiencing stretch perpendicular to strand alignment, cells not directly in contact with the strands aligned along the direction of stretch (up to 80%), whereas those in contact with strands remained aligned with the strands.[277] Interestingly, the cells that remained aligned with the strands increased MMP activity, possibly reflecting an attempt to remodel the matrix to equilibrate the new mechanical loads.[277] On grooved surfaces, stretch applied perpendicular to the direction of the groove similarly increases the degree of attached cell orientation along the grooves.[278] However, contact guidance stimuli are not necessarily the dominant influence on cell organization. For example, when the direction of fluid flow is not the same as the pattern of features (e.g., flow is perpendicular to the direction of grooves), contact guidance predominates under moderate

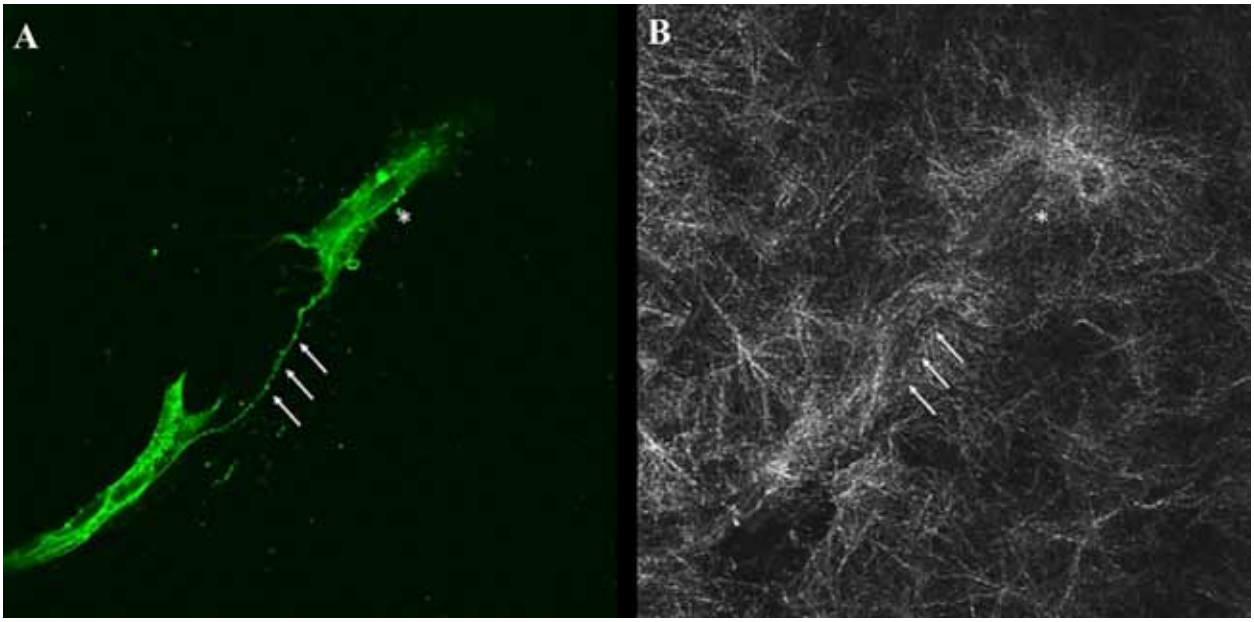

FIGURE 3. Matrix organization and microvascular angiogenesis. **A:** Filopodial extensions from the tips of 2 sprouting neovessels (green) forming a connection (arrows) coincident with organized collagen fibrils (**B**), as observed on reflectance confocal imaging. The asterisk serves as a marker to relate neovessel sprouts and collagen fibrils; projected 60× confocal image stacks).

shear whereas flow direction is predominant under high shear,[222] even though the actin cytoskeleton of the cell aligns in the direction of the grooves regardless of flow direction and extent.[222] In a similar way, ECs cultured on a micropatterned substrate made up of step changes in collagen density preferentially move to the areas of higher substrate density down the haptotaxis gradient.[185,186] However, application of a suprathreshold fluid shear in the opposite direction causes the cells to move into a region with lower substrate density.[185,279] A similar change in direction of cell migration could be generated by locally deforming the substrate at the leading or trailing edge of the migrating cell.[186] All of this suggests that while the cell prefers to follow the structure of underlying substrate, likely via focal adhesion complexes coupled to an organization cytoskeleton,[279] mechanical forces can superimpose some level of control.

While these examples highlight cell alignment and morphology responses to applied forces and structured substrates, these stimuli influence higher-order cellular organization. For instance, tenocytes cultured in stressed collagen I gels form an external sheath around the periphery, similar to what is observed in a native tendon, despite collagen microstructure.[2] The development of both parallel and perpendicular cellular processes formed by neuroblasts on microgrooved substrates is reminiscent of the neurite branching and organization of the *in vivo* tissue.[227] While mechanical forces influence the direction of growth of individual neovessels during angiogenesis,[66,238] they also seem to influence overall network topology.[60] Specific to the microvasculature, it may be that these different stimuli influence the cells to varying degrees during the angiogenesis and postangiogenesis programs (Fig. 4). For example, as the new sprout forms and advances, the tip cells orient ECM structure[65] via lamellipodia, and cell traction may lead to progressive alignment of the growing neovessels. The subsequent development of tension and hemodynamic forces related to blood flow would then influence vascular cell phenotype, leading to the formation and adaptation of a functional vascular network.[241] We are currently examining the temporal sequence

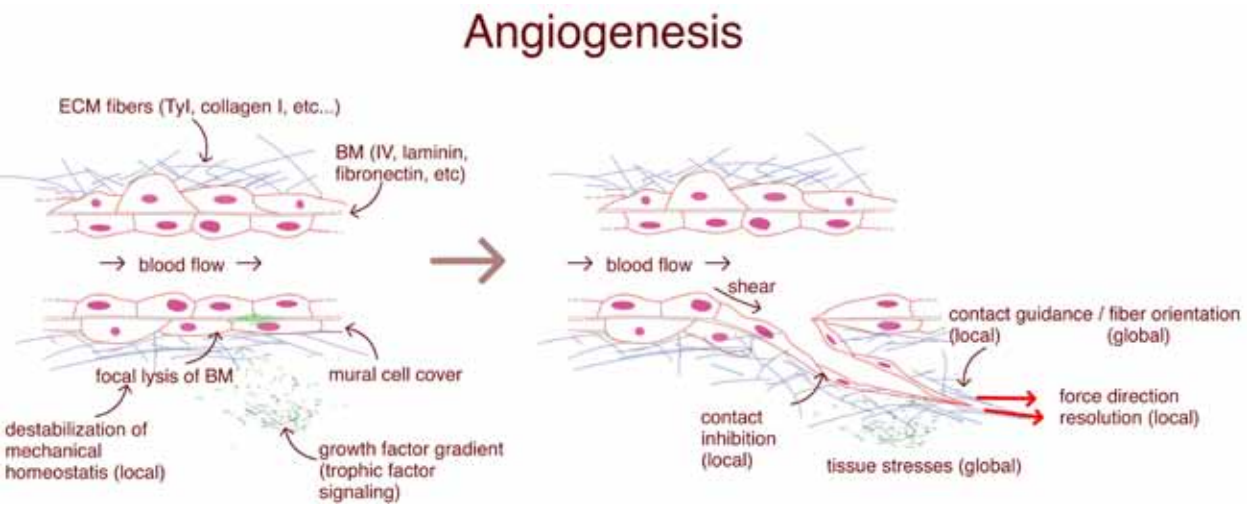

FIGURE 4. Schematic of the various stimuli influencing sprouting angiogenesis. Maintenance of vessels in quiescence, initiation and control of angiogenesis, and patterning of newly formed microvasculature depend on the integration of several inputs to the endothelial cell machinery that act locally and globally. BM – Basement membrane; ECM, extracellular matrix.

of events in neovessel orientation, matrix fibril orientation, and tissue loading to test this hypothesis.

D. Shear (Laminar and Interstitial Flow) Forces

The effects of fluid shear stress applied to the apical surface of ECs are extensive and varied. Exposure to laminar shear stress in culture promotes EC quiescence, alters gene expression, changes thrombotic potential, promotes an anti-inflammatory phenotype, and induces flow alignment.[280–282] Conditions of high shear rates, such as in turbulent or disrupted flow, tend to bring about the opposite effects.[282] In subconfluent cultures, migration is directed along the axis of shear stress.[279] When cultured on collagen I gels, applied shear stress promotes the invasion of ECs induced by growth factors.[126] In vessels, shear stress contributes significantly to vascular tone and structural adaptation via EC-dependent mediators.[56,283] Underlying substrates can alter EC responses to shear stress. For example, the presence of ECM dampened or differentially delayed responses to fluid shear stress.[284,285] Flow-dependent reorientation of ECs requires a concomitant change in the organization of the cytoskeleton.[286–288] In contrast, cells align perpendicular to the direction of large interstitial flows.[289] Interestingly, interstitial fluid flow stimulates vascular and lymphatic ECs differently than simple laminar fluid shear alone.[290]

V. PATTERNING VASCULAR CELLS

Unlike large-caliber vessels, where it is relatively easier to assemble the different layers of the vessel wall,[141] the formation of the microvasculature requires assembly of small-caliber vessels (often with walls of only 2 cell layers) into a complex, organized network. The formation of microvessels has generally involved the uncoordinated assembly of cultured vascular cells or vascular cell precursors to form capillary-like cell structures[291] or cell networks.[127,292] Few, but still successful, strategies have involved using native vessel elements such as microvessel fragments[31] or aortic rings.[293] While most past efforts have focused on manipulating the microenvironment constituents and cell compositions,[98,119,294–296] only recently has there been an interest in spatially directing neovessel assembly and formation in 2 and 3 dimensions. While many vascular cell systems have some ability to self-assemble, the neovessels and early networks that form may not reflect proper vessel wall structure or tissue architecture, delaying proper vascularization or leading

to network instability. Prepatterning the assembly of neovessels and networks might then lead to quicker and more successful implant integration and tissue health. In addition to using mechanical and contact guidance stimuli to control vascular geometry, other approaches are being used to physically direct vessel organization.

A. Microelectromechanical Systems–based Blood Vessels

A common approach to creating specific microvascular network geometries involves a combination of classic biological techniques with MEMS. Derived from the microelectronics industry, silicon wafers and photosensitive polymers are chemically manipulated to create planar patterns of channels.[297] Fabricated designs range from simple lines or geometric shapes[291] to complex interconnected networks.[298] Features within these patterns can range from nanometers to millimeters but usually reflect typical microvessel widths (5–200 μm). The typical approach involves the creation of a master mold, which is used to cast an elastomer,[299] composite polymer,[300] or biodegradable[301] replica of the pattern. By far, the most commonly used polymer in the molds for patterning microvascular cells[291,302,303] is the inert elastomer poly(dimethyl siloxane). However, biological polymers such as collagen,[304–306] laminin,[307] and fibronectin[308] can also be patterned by a mold generated by a MEMS. Cells then are placed within the patterned channels to form the microvessel elements with defined network geometries. Other MEMS or MEMS-like approaches include spraying cells through precise masks to form cell patterns on collagen films[309] and assembling "off-set printed" channel networks and cultured cell monolayers over the patterns.[310]

To manipulate vascular cell phenotype, channel surfaces have been chemically modified with solvents,[311] proteins,[312] or radiofrequency-generated plasma[302] to change surface-cell interactions. Taking advantage of the cellular responses to structured substrates, cell adhesion and growth also have been modified by manipulating surface micro-[313] and nanotopography.[314] In addition, researchers have expanded cell culture techniques by directing individual[315] and multiple cell types[316] to grow simultaneously on microfabricated surfaces in an effort to preassemble the microvessel wall. Other groups have worked to generate 3D layers of cells and ECM proteins in an attempt to replicate more complicated tissues, such as arterial walls.[317] We have used a MEMS approach to pattern angiogenic neovessels within defined geometries.[60,318] Continuing efforts to fabricate more complicated *in vitro* vessels and vessel networks, researchers have fabricated microchannels that shape biological proteins coated and/or seeded with microvascular cells.[305] In these systems, flowing media is used to introduce growth factor gradients, pressure gradients, and shear effects to *in vitro* cell cultures. EC monolayers as well as networks of capillary-like structures have been formed within collagen hydrogels. Evidence of microbeads lodged within lumen-like structures suggests these nascent structures may form capillary-like structures that may be perfused via nearby microchannels. Similar network structures have been formed in multicellular cultures.[319] These results suggest that more complicated tissue mimics containing vessels may be possible in the near future.

B. Bioprinting Microvasculatures

In addition to "top down" MEMS microfabrication, a second class of technologies focuses on "bottom up" fabrication and direct incorporation of cells within 3D structures via printing of cells and substrates ("bio-printing").[320] While MEMS approaches are very effective at forming complex planar systems, it is difficult to build complex patterns in 3 dimensions.[321] The first efforts at bio-printing involved inkjet printers to dispense cells onto substrates.[322] Inkjet cartridges and nozzles, modified to accommodate cells, dispense droplets of polymer suspensions or cell-polymer suspensions onto a surface. In a computer-controlled, layer-by-layer fashion, cells and proteins can be deposited onto various substrates, including glass, collagen, agar, and Matrigel, forming simple geometric shapes.[323] More complex geometries recently have been printed with multiple cells encapsulated within hydrogels.[324] An alternate system for fabricating 3D geometries

is direct-write bio-printing.[325] In contrast to inkjet cartridges and tubing, direct-write bio-printing uses syringes to directly dispense (typically via servo or pneumatic pressure) biologicals into 3D structures.[326] Similar to solid freeform fabrication, complex shapes are constructed layer by layer. Both direct-write and inkjet bio-printing can organize cells and polymers onto substrates. However, a major advantage of direct-write printing is a greater range of printable viscosities. For instance, we routinely print 40 wt% Pluronic F127 and biological suspensions of 3 mg/mL collagen, which are about 600×10^6 mPa · s. Direct-write printing recently has been used to form individual microvessels. By printing stacking rings of cell "spheroids,"[326] small, cellularized tubes have been constructed. Likewise, linear and branched planar, vessel-like structures also have been fabricated.[327]

VI. COMPUTATIONAL MODELING OF THE MICROVASCULATURE

Progress in microvascular engineering has resulted in several strategies for creating microvascular networks, primarily *in vitro*, and some have focused on imposing topological features. Evidence of vessel maturation, pruning, phenotypic changes to match local vascular beds, and topological adaptations all point clearly to the existence of complex regulators that are highly integrated spatiotemporally and difficult to separate experimentally into cause and effect models.[36,60,116,328] Thus, computationally modeling these networks can offer interesting insights into these processes and provide new experimental directions to understand the factors involved in both the formation and maintenance of the microvascular bed and the development of novel pharmaceutical targets that influence the process of angiogenesis. In general, models of angiogenesis range in complexities from a focus on cell-level interactions with respect to biochemical variables to macrolevel biophysical dynamics. However, the interdependence of several factors that are considered to be driving forces behind angiogenesis and vascular patterning make this a nontrivial problem both experimentally and computationally. This review addresses only the role of computational modeling in the context of its impact on our understanding of effectors of microvascular topology and morphology.

A. Determinants of Microvascular Branching and Morphology

The development of microvascular branching patterns and network topologies in various tissue beds is not well understood. Over the past decade, several "rules" guiding the growth and maturation of a microvascular network have been put forth. Hansen-Smith[329] proposed that capillary network patterns can be determined by the same mechanisms in all tissues but have tissue- and stimulus-specific regulators. Using symmetric bifurcation of each generation of vessels, with the parent and daughter vessel diameters related by Murray's law, as a rule has yielded a basic model of branched microvasculature.[242] Other models of vascular network branching and diameters were proposed based on the idea of *minimum work*.[14,80] In addition, that the vasculatures and their variations can be described by their fractal properties,[8,10] with dimensional values ranging from 1.70 in muscle to 1.89 in the kidney, may be indicative of a conserved and iterative branch morphogenesis mechanism.[8,10,242,330] While there are likely many molecular determinants, which are possibly developmentally preprogrammed,[330] final branching patterns likely arise from secondary stimuli such as matrix orientation, matrix mechanics, tissue oxygen consumption, and hemodynamic influences superimposed on the genetic programs.

B. Angiogenesis

Given the complexity of angiogenesis with neovascular cells continuously responding to and influencing pro- and antiangiogenesis signals, matrix structure and composition, both chemo- and haptotactical gradients, and extravascular cell dynamics, computational models have proven useful in describing the relevant integrated behaviors and making predictions as to underlying mechanisms. Often multiscaled,[331] such models (discretized and/or continuous) integrate biochemical, cellular, and ECM dynamics to predict the extent, direction, and

progression of neovessel growth. Such models are particularly effective at simultaneously describing emergent neovessel behaviors across temporal and spatial domains. Many efforts at describing angiogenesis via computational models have focused on tumor angiogenesis, often involving EC migration and secreted angiogenic factors acting as chemotactic agents.[332–337] As more experimental findings are described, the spectrum of computational models simulating neovessel sprouting and behavior have increased (see Refs. 338–340). For example, the behavior of tip and stalk cells within a sprouting neovessel have been developed by building off of the growing experimental evidence describing this interesting dynamic and the signaling axis of VEGF-Notch-DII4.[341] Also, VEGF dynamics involving both matrix-bound and more freely diffusible forms have been modeled to capture the impact of the varied VEGF regulatory mechanisms on an overall angiogenesis response.[342]

Computational models of angiogenesis indicate that the ECM plays a strong role in neovessel sprouting and elongation beyond simply providing a substrate for cell adhesion and migration. In a tumor angiogenesis model involving secreted angiogenic factors and based on a cell-centric approach, matrix dynamics was predicted to significantly affect neovessel branching and anastomosis.[343] In a model of collagen matrix deformation, neovessel alignment and morphology during angiogenesis similarly depended on the extent and axis of strain, which correlated with collagen fibril orientation.[238] An interesting collective prediction from a variety of models is that angiogenesis (and the subsequent postangiogenesis phase) depends on both deterministic and stochastic processes. For example, in a model of network expansion via angiogenesis, an overabundance of new vessels formed stochastically accompanied by network refinement via structural adaptation and pruning through deterministic "rules."[344] More and more, investigators are creating computational models in which angiogenesis-related phenomena are integrated with those related to vessel adaptation and network maturation over a larger temporal and spatial scale.

C. Microvascular Structural Adaptation

Several modeling approaches exist that utilize the relationship between flow shear, circumferential stretch, hoop stresses, vessel diameters, interstitial flow, metabolites and chemokines, and upstream and downstream conductive responses of these stimuli along the microvessels to predict the structural adaptation of networks (see Ref. 345). In an interesting report, Pries and Secomb[346] showed that local heterogeneous blood flow and metabolic patterns have only limited dependence on the oxygen demand, suggesting that local metabolic needs adapt to the availability of oxygen. Reglin et al.[347] used a novel advection and diffusion–coupled model to enhance the distribution of oxygen in the tissue space, rather than the more simplistic axially distributed models, and demonstrated adequate oxygenation of cardiac tissue for physiologic rates of flow and consumption in spite of assuming a microvasculature more characteristic of skeletal tissue. Godde et al.[348] included vessel regression and for the first time simulated the interdigitations between terminal components of arterial and venous trees; they also showed a higher influence of shear-driven dynamics rather than pressure-driven dynamics on the occurrence of these interdigitations and that pressure-driven systems tend to progress toward "arterialization" of the network, with almost total absence of the venous parts. The role of interstitial flow as a significant regulator of the microvascular morphology is gradually being recognized. Even subtle changes in interstitial flow can alter the chemotactic gradient of extracellular molecules in the microenvironment, mainly that of the convection-driven macromolecules and proteins rather than solutes such as oxygen, which are dependent on diffusion.[349] In addition to surface fluid shear, interstitial flow also is responsible for changes in the mechanical microenvironment or redistribution of chemokines. Using a fluid dynamics model, Pedersen et al.[350] predicted that the perpendicular alignment of matrix fibers to interstitial flow shields cells from forces such as shear and pressure. Jain et al.[351] used a mathematical model to address the interstitial pressures and fluid velocity of tumors; they reconciled several related observations pertaining to anticancer therapy to explain the relationship between normalization of tumor vasculature

and reduced tumor metastasis and other beneficial effects.[19] These findings are congruous with subsequent conclusions by Pries et al.,[242] who demonstrated that subjecting normal microvasculature to parameters of a tumor network led them to exhibit tumor vasculature–like properties.

D. Modeling the Matrix Environment and Cell-Matrix Interactions

Artificial matrices, mostly composed of a hydrogel substrate, have been assigned a biphasic nature comprising a continuous fibrillar architecture interspersed with interstitial fluid.[250] The intraphase, viscoelastic nature of such substrates is attributable to a bulk phase fiber-fluid interaction component and an intrinsic viscoelastic component of the fiber-forming protein structure, such as collagen I.[250,352] Using finite element analysis, the cell traction force from substrate deformations can be estimated and used as an input to determine the work done by the cell or its strain energy function, assuming an isotropic linear elastic substrate.[184,186] Many cellular modeling and fiber reorientation studies are based on the results from matrices seeded with fibroblasts,[77,214,251,252,276,353,354] but ECs may interact differently with the ECM, especially as a part of the tubular microvascular structure. Models of tubulogenesis driven under cell traction fields suggest that the amplitude of strain field is too weak to influence tubulogenesis for conditions leading to mechanical remodeling of the ECM.[355] Thus it may be inferred that although directional cell migration occurs in a strain field, it may not be essential in the native tubulogenesis process.[32,82,355]

Modeling the interactions of growing microvascular networks and the microenvironment presents significant challenges because of the heterogeneity of cell types, varying vessel morphology, and the need to integrate several competing stimuli into a set of concerted responses. Guilkey et al.[356] used a meshless method, called the material point method, to predict the forces and deformations at the growing tip of capillary sprouts. Here, a 3D particle representation of the complex network was created by converting volume-rendered confocal image stacks into discrete particles, with the all relevant information carried on the particles themselves; calculations were performed on a background grid or scratchpad. Using global values for mechanical properties of the construct, the model revealed an inhomogeneous stress distribution around the cellular elements, even in uniaxial tensile loading, demonstrating the efficacy of this method in studying the interactions between microvessels and its matrix environment. More recent models using a "controller" to provide a platform for integration and exchange of parameters between existing modules have been proposed.[331] This approach successfully predicted the effects of migration separate from proliferation on the tip cell and stalk cell movement driven both by a VEGF chemotactic gradient and a push-pull relationship between the sprouting tip and the following stalk cells differentially in a temporal event sequence.[357]

VII. SUMMARY AND FUTURE CONSIDERATIONS

It is now well accepted that manipulation of tissues, either to repair native tissues or build engineered tissues, must include a strategy establishing a functional vasculature. In tissue construction, where there is an absence of existing vessels, this strategy is primarily based on providing the cellular building blocks necessary for assembling/forming vessel elements. Coupled with both diffusible and solid-state factors that stimulate these vessel precursors, many strategies effectively form new vessel elements in a variety of tissue spaces and applications. New investigations into the affect of the microenvironment on vascularization outcomes indicate that the extravascular matrix and cellular environment significantly affects vascular character through processes acting coordinately with the biochemical signals intrinsic to these elements. Matrix microarchitecture, deformation, and mutability all influence the extent of neovessel growth, guidance, and patterning. Because of this growing complexity, many approaches used to understand angiogenesis in constructed and native tissues are combining computational and experimental strategies to discern mechanistic underpinnings and system-wide dynamics.

While much of the focus to date has been on forming new vessels (both macro- and microves-

sels), it is becoming clear that organization and performance of the vascular network are equally important. In native tissues, vascular organization complements tissue organization, leading to healthy tissue function. Furthermore, proper microvascular function depends on the assembly of individual microvessels into an effective network and perfusion circuit. Much is known concerning the mechanisms of vascular growth. However, further work is needed to understand how new vessels are patterned into a vascular tree, the roles contact guidance and force guidance play in vascular morphology, and the integrative nature of global and local control in structuring vascular beds. As more is learned about the mechanisms underlying not only angiogenesis but also vascular patterning and network adaptation, it should be possible to manipulate the microvasculature to achieve the desired therapeutic outcome.

ACKNOWLEDGMENTS

Supported by National Institutes of Health grants HL077683 (J.A.W.), DK078175 (S.K.W.), and EB007556 (J.B.H.).

REFERENCES

1. Evers R, Khait L, Birla RK. Fabrication of functional cardiac, skeletal, and smooth muscle pumps *in vitro*. Artif Organs. 2011;35(1):69–74.
2. Garvin J, Qi J, Maloney M, Banes AJ. Novel system for engineering bioartificial tendons and application of mechanical load. Tissue Eng. 2003;9(5):967–79.
3. Yamamoto Y, Ito A, Kato M, Kawabe Y, Shimizu K, Fujita H, Nagamori E, Kamihira M. Preparation of artificial skeletal muscle tissues by a magnetic force-based tissue engineering technique. J Biosci Bioeng. 2009;108(6):538–43.
4. Yang D, Guo T, Nie C, Morris SF. Tissue-engineered blood vessel graft produced by self-derived cells and allogenic acellular matrix: a functional performance and histologic study. Ann Plast Surg. 2009;62(3):297–303.
5. Hattori K, Joraku A, Miyagawa T, Kawai K, Oyasu R, Akaza H. Bladder reconstruction using a collagen patch prefabricated within the omentum. Int J Urol. 2006;13(5):529–37.
6. Bouhadir KH, Mooney DJ. Promoting angiogenesis in engineered tissues. J Drug Target. 2001;9(6):397–406.
7. Mann BK, West JL. Tissue engineering in the cardiovascular system: progress toward a tissue engineered heart. Anat Rec. 2001;263(4):367–71.
8. Andreadis ST. Gene transfer to epidermal stem cells: implications for tissue engineering. Expert Opin Biol Ther. 2004;4(6):783–800.
9. Folkman J, Hochberg M. Self-regulation of growth in three dimensions. J Exp Med. 1973;138(4):745–53.
10. Chaikof EL. Engineering and material considerations in islet cell transplantation. Annu Rev Biomed Eng. 1999;1:103–27.
11. Cassell OC, Hofer SO, Morrison WA, Knight KR. Vascularisation of tissue-engineered grafts: the regulation of angiogenesis in reconstructive surgery and in disease states. Br J Plast Surg. 2002;55(8):603–10.
12. Okano T, Matsuda T. Muscular tissue engineering: capillary-incorporated hybrid muscular tissues *in vivo* tissue culture. Cell Transplant. 1998;7(5):435–42.
13. Ko HC, Milthorpe BK, McFarland CD. Engineering thick tissues–the vascularisation problem. Eur Cells Mater. 2007;14:1–18; discussion 18–9.
14. Eiselt P, Kim BS, Chacko B, Isenberg B, Peters MC, Greene KG, Roland WD, Loebsack AB, Burg KJ, Culberson C, Halberstadt CR, Holder WD, Mooney DJ. Development of technologies aiding large-tissue engineering. Biotechnol Prog. 1998;14(1):134–40.
15. Soker S, Machado M, Atala A. Systems for therapeutic angiogenesis in tissue engineering. World J Urol. 2000;18(1):10–8.
16. Traktuev DO, Prater DN, Merfeld-Clauss S, Sanjeevaiah AR, Saadatzadeh MR, Murphy M, Johnstone BH, Ingram DA, March KL. Robust functional vascular network formation *in vivo* by cooperation of adipose progenitor and endothelial cells. Circ Res. 2009;104(12):1410–20.
17. Fumimoto Y, Matsuyama A, Komoda H, Okura H, Lee CM, Nagao A, Nishida T, Ito T, Sawa Y. Creation of a rich subcutaneous vascular network with implanted adipose tissue-derived stromal cells and adipose tissue enhances subcutaneous grafting of islets in diabetic mice. Tissue Eng Part C Methods. 2009;15(3):437–44.
18. Seandel M, Noack-Kunnmann K, Zhu D, Aimes RT, Quigley JP. Growth factor-induced angiogenesis *in vivo* requires specific cleavage of fibrillar type I collagen. Blood. 2001;97(8):2323–32.
19. Shepherd BR, Chen HY, Smith CM, Gruionu G, Williams SK, Hoying JB. Rapid perfusion and network remodeling in a microvascular construct after implantation. Arterioscler Thromb Vasc Biol. 2004;24(5):898–904.
20. Shepherd BR, Hoying JB, Williams SK. Microvascular transplantation after acute myocardial infarction. Tissue Eng. 2007;13(12):2871–9.
21. Shimizu T, Sekine H, Yang J, Isoi Y, Yamato M, Kikuchi A, Kobayashi E, Okano T. Polysurgery of cell sheet grafts overcomes diffusion limits to produce thick, vascularized myocardial tissues. FASEB J. 2006;20(6):708–10.
22. Schechner JS, Nath AK, Zheng L, Kluger MS, Hughes CC, Sierra-Honigmann MR, Lorber MI, Tellides G, Kashgarian M, Bothwell AL, Pober JS. *In vivo* formation of

complex microvessels lined by human endothelial cells in an immunodeficient mouse. Proc Natl Acad Sci U S A. 2000;97(16):9191–6.
23. Neumann T, Nicholson BS, Sanders JE. Tissue engineering of perfused microvessels. Microvasc Res. 2003;66(1):59–67.
24. Nor JE, Peters MC, Christensen JB, Sutorik MM, Linn S, Khan MK, Addison CL, Mooney DJ, Polverini PJ. Engineering and characterization of functional human microvessels in immunodeficient mice. Lab Invest. 2001;81(4):453–63.
25. Novosel EC, Kleinhans C, Kluger PJ. Vascularization is the key challenge in tissue engineering. Adv Drug Deliv Rev. 2011;63(4–5):300–11.
26. Phelps EA, Garcia AJ. Engineering more than a cell: vascularization strategies in tissue engineering. Curr Opin Biotechnol. 2010;21(5):704–9.
27. Pittman RN. Oxygen transport and exchange in the microcirculation. Microcirculation. 2005;12(1):59–70.
28. Risau W. Development and differentiation of endothelium. Kidney Int Suppl. 1998;67:S3–6.
29. Jain RK. Molecular regulation of vessel maturation. Nat Med. 2003;9(6):685–93.
30. Shiu YT, Weiss JA, Hoying JB, Iwamoto MN, Joung IS, Quam CT. The role of mechanical stresses in angiogenesis. Crit Rev Biomed Eng. 2005;33(5):431–510.
31. Hoying JB, Boswell CA, Williams SK. Angiogenic potential of microvessel fragments established in three-dimensional collagen gels. *In Vitro* Cell Dev Biol Anim. 1996;32(7):409–19.
32. Vernon RB, Lara SL, Drake CJ, Iruela-Arispe ML, Angello JC, Little CD, Wight TN, Sage EH. Organized type I collagen influences endothelial patterns during "spontaneous angiogenesis *in vitro*": planar cultures as models of vascular development. *In Vitro* Cell Dev Biol Anim. 1995;31(2):120–31.
33. Madri JA, Williams SK. Capillary endothelial cell cultures: phenotypic modulation by matrix components. J Cell Biol. 1983;97(1):153–65.
34. Risau W. Differentiation of endothelium. FASEB J. 1995;9(10):926–33.
35. Bergers G, Song S. The role of pericytes in blood-vessel formation and maintenance. Neuro Oncol. 2005;7(4):452–64.
36. Nunes SS, Greer KA, Stiening CM, Chen HY, Kidd KR, Schwartz MA, Sullivan CJ, Rekapally H, Hoying JB. Implanted microvessels progress through distinct neovascularization phenotypes. Microvasc Res. 2010;79(1):10–20.
37. Zakrzewicz A, Secomb TW, Pries AR. Angioadaptation: keeping the vascular system in shape. News Physiol Sci. 2002;17:197–201.
38. Risau W. Mechanisms of angiogenesis. Nature. 1997;386(6626):671–4.
39. Marti HH, Risau W. Angiogenesis in ischemic disease. Thromb Haemost. 1999;82(Suppl 1):44–52.
40. Aitsebaomo J, Portbury AL, Schisler JC, Patterson C. Brothers and sisters: molecular insights into arterial-venous heterogeneity. Circ Res. 2008;103(9):929–39.
41. Schmid-Schonbein GW, Skalak TC, Firestone G. The microvasculature in skeletal muscle. V. The microvascular arcades in normotensive and hypertensive rats. Microvasc Res. 1987;34(3):385–93.
42. Tomanek RJ, Hansen HK, Dedkov EI. Vascular patterning of the quail coronary system during development. Anat Rec A Discov Mol Cell Evol Biol. 2006;288(9):989–99.
43. Vasudevan A, Long JE, Crandall JE, Rubenstein JL, Bhide PG. Compartment-specific transcription factors orchestrate angiogenesis gradients in the embryonic brain. Nat Neurosci. 2008;11(4):429–39.
44. Isogai S, Lawson ND, Torrealday S, Horiguchi M, Weinstein BM. Angiogenic network formation in the developing vertebrate trunk. Development. 2003;130(21):5281–90.
45. Neufeld G, Cohen T, Shraga N, Lange T, Kessler O, Herzog Y. The neuropilins: multifunctional semaphorin and VEGF receptors that modulate axon guidance and angiogenesis. Trends Cardiovasc Med. 2002;12(1):13–9.
46. Metzger RJ, Krasnow MA. Genetic control of branching morphogenesis. Science. 1999;284(5420):1635–9.
47. Jones EA, le Noble F, Eichmann A. What determines blood vessel structure? Genetic prespecification vs. hemodynamics. Physiology (Bethesda). 2006;21:388–95.
48. Pruett ND, Visconti RP, Jacobs DF, Scholz D, McQuinn T, Sundberg JP, Awgulewitsch A. Evidence for Hox-specified positional identities in adult vasculature. BMC Dev Biol. 2008;8:93.
49. Gaengel K, Genove G, Armulik A, Betsholtz C. Endothelial-mural cell signaling in vascular development and angiogenesis. Arterioscler Thromb Vasc Biol. 2009;29(5):630–8.
50. Hellstrom M, Gerhardt H, Kalen M, Li X, Eriksson U, Wolburg H, Betsholtz C. Lack of pericytes leads to endothelial hyperplasia and abnormal vascular morphogenesis. J Cell Biol. 2001;153(3):543–53.
51. Krishnan L, Hoying JB, Nguyen H, Song H, Weiss JA. Interaction of angiogenic microvessels with the extracellular matrix. Am J Physiol Heart Circ Physiol. 2007;293(6):H3650–8.
52. Li J, Zhang YP, Kirsner RS. Angiogenesis in wound repair: angiogenic growth factors and the extracellular matrix. Microsc ResTech. 2003;60(1):107–14.
53. Ingber DE. Mechanical signaling and the cellular response to extracellular matrix in angiogenesis and cardiovascular physiology. Circ Res. 2002;91(10):877–87.
54. Norman JJ, Collins JM, Sharma S, Russell B, Desai TA. Microstructures in 3D biological gels affect cell proliferation. Tissue Eng Part A. 2008;14(3):379–90.
55. Rocha SF, Adams RH. Molecular differentiation and specialization of vascular beds. Angiogenesis. 2009;12(2):139–47.

56. Pries AR, Reglin B, Secomb TW. Remodeling of blood vessels: responses of diameter and wall thickness to hemodynamic and metabolic stimuli. Hypertension. 2005;46(4):725–31.
57. Coultas L, Nieuwenhuis E, Anderson GA, Cabezas J, Nagy A, Henkelman RM, Hui CC, Rossant J. Hedgehog regulates distinct vascular patterning events through VEGF dependent and independent mechanisms. Blood. 2010;116(4):653–60.
58. Chang CC, Krishnan L, Reese S, Boland ED, Williams SK, Hoying JB. *In Vitro* Patterned Microvessels Lose Alignment *In Vivo*. 9th World Congress for Microcirculation, Paris, France September 2010.
59. Krishnan L, Chang CC, Reese SP, Williams SK, Weiss JA, Hoying JB. Anchorage dependent persistent alignment of perfused microvasculature in implanted tissue constructs. Proceedings of the ASME Summer Bioengineering Conference; 2011 June 22–25; Farmington, PA.
60. Chang CC, Krishnan L, Nunes SS, Church KH, Edgar LT, Boland ED, Weiss JA, Williams SK, Hoying JB. Determinants of microvascular network topologies in implanted neovasculatures. Arterioscler Thromb Vasc Biol. 2012;32(1):5–14.
61. Haas TL, Davis SJ, Madri JA. Three-dimensional type I collagen lattices induce coordinate expression of matrix metalloproteinases MT1-MMP and MMP-2 in microvascular endothelial cells. J Biol Chem. 1998;273(6):3604–10.
62. Abramsson A, Lindblom P, Betsholtz C. Endothelial and nonendothelial sources of PDGF-B regulate pericyte recruitment and influence vascular pattern formation in tumors. J Clin Invest. 2003;112(8):1142–51.
63. Leveen P, Pekny M, Gebre-Medhin S, Swolin B, Larsson E, Betsholtz C. Mice deficient for PDGF B show renal, cardiovascular, and hematological abnormalities. Genes Dev. 1994;8(16):1875–87.
64. Evensen L, Micklem DR, Blois A, Berge SV, Aarsaether N, Littlewood-Evans A, Wood J, Lorens JB. Mural cell associated VEGF is required for organotypic vessel formation. PLoS One. 2009;4(6):e5798.
65. Kirkpatrick ND, Andreou S, Hoying JB, Utzinger U. Live imaging of collagen remodeling during angiogenesis. Am J Physiol Heart Circ Physiol. 2007;292(6):H3198–206.
66. Krishnan L, Underwood CJ, Maas S, Ellis BJ, Kode TC, Hoying JB, Weiss JA. Effect of mechanical boundary conditions on orientation of angiogenic microvessels. Cardiovasc Res. 2008;78(2):324–32.
67. Distler JH, Hirth A, Kurowska-Stolarska M, Gay RE, Gay S, Distler O. Angiogenic and angiostatic factors in the molecular control of angiogenesis. Q J Nucl Med. 2003;47(3):149–61.
68. Gerhardt H, Golding M, Fruttiger M, Ruhrberg C, Lundkvist A, Abramsson A, Jeltsch M, Mitchell C, Alitalo K, Shima D, Betsholtz C. VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. J Cell Biol. 2003;161(6):1163–77.
69. Wang H, Keiser JA. Vascular endothelial growth factor upregulates the expression of matrix metalloproteinases in vascular smooth muscle cells: role of flt-1. Circ Res. 1998;83(8):832–40.
70. Kim J, Oh WJ, Gaiano N, Yoshida Y, Gu C. Semaphorin 3E-Plexin-D1 signaling regulates VEGF function in developmental angiogenesis via a feedback mechanism. Genes Dev. 2011;25(13):1399–411.
71. Affolter M, Zeller R, Caussinus E. Tissue remodelling through branching morphogenesis. Nat Rev Mol Cell Biol. 2009;10(12):831–42.
72. Ehrbar M, Metters A, Zammaretti P, Hubbell JA, Zisch AH. Endothelial cell proliferation and progenitor maturation by fibrin-bound VEGF variants with differential susceptibilities to local cellular activity. J Control Release. 2005;101(1–3):93–109.
73. Peters MC, Polverini PJ, Mooney DJ. Engineering vascular networks in porous polymer matrices. J Biomed Mater Res. 2002;60(4):668–78.
74. Griffith CK, George SC. The effect of hypoxia on *in vitro* prevascularization of a thick soft tissue. Tissue Eng Part A. 2009;15(9):2423–34.
75. Swartz MA, Tschumperlin DJ, Kamm RD, Drazen JM. Mechanical stress is communicated between different cell types to elicit matrix remodeling. Proc Natl Acad Sci U S A. 2001;98(11):6180–5.
76. Haudenschild CC, Zahniser D, Folkman J, Klagsbrun M. Human vascular endothelial cells in culture. Lack of response to serum growth factors. Exp Cell Res. 1976;98(1):175–83.
77. Ingber DE. Fibronectin controls capillary endothelial cell growth by modulating cell shape. Proc Natl Acad Sci U S A. 1990;87(9):3579–83.
78. Ingber D. Extracellular matrix and cell shape: potential control points for inhibition of angiogenesis. J Cell Biochem. 1991;47(3):236–41.
79. Ingber D. Integrins as mechanochemical transducers. Curr Opin Cell Biol. 1991;3(5):841–8.
80. Gonen-Wadmany M, Gepstein L, Seliktar D. Controlling the cellular organization of tissue-engineered cardiac constructs. Ann N Y Acad Sci. 2004;1015:299–311.
81. Kolodney MS, Wysolmerski RB. Isometric contraction by fibroblasts and endothelial cells in tissue culture: a quantitative study. J Cell Biol. 1992;117(1):73–82.
82. Korff T, Augustin HG. Tensional forces in fibrillar extracellular matrices control directional capillary sprouting. J Cell Sci. 1999;112(Pt 19):3249–58.
83. Ingber DE. Mechanical signaling and the cellular response to extracellular matrix in angiogenesis and cardiovascular physiology. Circ Res. 2002;91(10):877–87.
84. Wang M, Su Y, Sun H, Wang T, Yan G, Ran X, Wang F, Cheng T, Zou Z. Induced endothelial differentiation of cells from a murine embryonic mesenchymal cell line C3H/10T1/2 by angiogenic factors *in vitro*. Differentiation. 2010;79(1):21–30.

85. Kinoshita K, Tanjoh K, Noda A, Sakurai A, Yamaguchi J, Azuhata T, Utagawa A, Moriya T. Interleukin-8 production from human umbilical vein endothelial cells during brief hyperglycemia: the effect of tumor necrotic factor-alpha. J Surg Res. 2008;144(1):127–31.
86. Stuttfeld E, Ballmer-Hofer K. Structure and function of VEGF receptors. IUBMB Life. 2009;61(9):915–22.
87. Lai TS, Liu Y, Li W, Greenberg CS. Identification of two GTP-independent alternatively spliced forms of tissue transglutaminase in human leukocytes, vascular smooth muscle, and endothelial cells. FASEB J. 200721(14):4131–43.
88. Thomas M, Augustin HG. The role of the angiopoietins in vascular morphogenesis. Angiogenesis. 2009;12(2):125–37.
89. Bagley RG, Weber W, Rouleau C, Teicher BA. Pericytes and endothelial precursor cells: cellular interactions and contributions to malignancy. Cancer Res. 2005;65(21):9741–50.
90. Kutcher ME, Herman IM. The pericyte: cellular regulator of microvascular blood flow. Microvasc Res. 2009;77(3):235–46.
91. Nourse MB, Halpin DE, Scatena M, Mortisen DJ, Tulloch NL, Hauch KD, Torok-Storb B, Ratner BD, Pabon L, Murry CE. VEGF induces differentiation of functional endothelium from human embryonic stem cells: implications for tissue engineering. Arterioscler Thromb Vasc Biol. 2010;30(1):80–9.
92. Pompe T, Markowski M, Werner C. Modulated fibronectin anchorage at polymer substrates controls angiogenesis. Tissue Eng. 2004;10(5–6):841–8.
93. Chen B, Dang J, Tan TL, Fang N, Chen WN, Leong KW, Chan V. Dynamics of smooth muscle cell deadhesion from thermosensitive hydroxybutyl chitosan. Biomaterials. 2007;28(8):1503–14.
94. Ingber DE, Folkman J. Mechanochemical switching between growth and differentiation during fibroblast growth factor-stimulated angiogenesis *in vitro*: role of extracellular matrix. J Cell Biol. 1989;109(1):317–30.
95. Reed MJ, Karres N, Eyman D, Vernon RB. Culture of murine aortic explants in 3-dimensional extracellular matrix: a novel, miniaturized assay of angiogenesis *in vitro*. Microvasc Res. 2007;73(3):248–52.
96. Bellacen K, Lewis EC. Aortic ring assay. J Vis Exp. 2009;(33). pii: 1564.
97. Nicosia RF, Zhu WH, Fogel E, Howson KM, Aplin AC. A new *ex vivo* model to study venous angiogenesis and arterio-venous anastomosis formation. J Vasc Res. 2005;42(2):111–9.
98. Yang J, Nagavarapu U, Relloma K, Sjaastad MD, Moss WC, Passaniti A, Herron GS. Telomerized human microvasculature is functional *in vivo*. Nat Biotechnol. 2001;19(3):219–24.
99. Shepherd BR, Enis DR, Wang F, Suarez Y, Pober JS, Schechner JS. Vascularization and engraftment of a human skin substitute using circulating progenitor cell-derived endothelial cells. FASEB J. 2006;20(10):1739–41.
100. Darland DC, D'Amore PA. TGF beta is required for the formation of capillary-like structures in three-dimensional cocultures of 10T1/2 and endothelial cells. Angiogenesis. 2001;4(1):11–20.
101. Nehls V, Drenckhahn D. A novel, microcarrier-based *in vitro* assay for rapid and reliable quantification of three-dimensional cell migration and angiogenesis. Microvasc Res. 1995;50(3):311–22.
102. Kniazeva E, Putnam AJ. Endothelial cell traction and ECM density influence both capillary morphogenesis and maintenance in 3-D. Am J Physiol Cell Physiol. 2009;297(1):C179–87.
103. Dietrich F, Lelkes PI. Fine-tuning of a three-dimensional microcarrier-based angiogenesis assay for the analysis of endothelial-mesenchymal cell co-cultures in fibrin and collagen gels. Angiogenesis. 2006;9(3):111–25.
104. Crabtree B, Subramanian V. Behavior of endothelial cells on Matrigel and development of a method for a rapid and reproducible *in vitro* angiogenesis assay. *In Vitro* Cell Dev Biol Anim. 2007;43(2):87–94.
105. Frerich B, Zuckmantel K, Winter K, Muller-Durwald S, Hemprich A. Maturation of capillary-like structures in a tube-like construct in perfusion and rotation culture. Int J Oral Maxillofac Surg. 2008;37(5):459–66.
106. Fleming PA, Argraves WS, Gentile C, Neagu A, Forgacs G, Drake CJ. Fusion of uniluminal vascular spheroids: a model for assembly of blood vessels. Dev Dyn. 2010;239(2):398–406.
107. Thakar RG, Cheng Q, Patel S, Chu J, Nasir M, Liepmann D, Komvopoulos K, Li S. Cell-shape regulation of smooth muscle cell proliferation. Biophys J. 2009;96(8):3423–32.
108. Churchman AT, Siow RC. Isolation, culture and characterisation of vascular smooth muscle cells. Methods Mol Biol. 2009;467:127–38.
109. Valarmathi MT, Davis JM, Yost MJ, Goodwin RL, Potts JD. A three-dimensional model of vasculogenesis. Biomaterials. 2009;30(6):1098–112.
110. Silva EA, Kim ES, Kong HJ, Mooney DJ. Material-based deployment enhances efficacy of endothelial progenitor cells. Proc Natl Acad Sci U S A. 2008;105(38):14347–52.
111. Kurzen H, Manns S, Dandekar G, Schmidt T, Pratzel S, Kraling BM. Tightening of endothelial cell contacts: a physiologic response to cocultures with smooth-muscle-like 10T1/2 cells. J Invest Dermatol. 2002;119(1):143–53.
112. Levenberg S, Rouwkema J, Macdonald M, Garfein ES, Kohane DS, Darland DC, Marini R, van Blitterswijk CA, Mulligan RC, D'Amore PA, Langer R. Engineering vascularized skeletal muscle tissue. Nat Biotechnol. 2005;23(7):879–84.
113. Goodwin AM. *In vitro* assays of angiogenesis for assessment of angiogenic and anti-angiogenic agents. Microvasc Res. 2007;74(2–3):172–83.
114. Auerbach R, Lewis R, Shinners B, Kubai L, Akhtar N. Angiogenesis assays: a critical overview. Clin Chem. 2003;49(1):32–40.

115. Laschke MW, Vollmar B, Menger MD. Inosculation: connecting the life-sustaining pipelines. Tissue Eng Part B Rev. 2009;15(4):455–65.
116. Nunes SS, Krishnan L, Gerard CS, Dale JR, Maddie MA, Benton RL, Hoying JB. Angiogenic potential of microvessel fragments is independent of the tissue of origin and can be influenced by the cellular composition of the implants. Microcirculation. 2010;17(7):557–67.
117. Frerich B, Zueckmantel K, Hemprich A. Microvascular engineering in perfusion culture: immunohistochemistry and CLSM findings. Head Face Med. 2006;2(1):26.
118. Takei T, Sakai S, Ono T, Ijima H, Kawakami K. Fabrication of endothelialized tube in collagen gel as starting point for self-developing capillary-like network to construct three-dimensional organs *in vitro*. Biotechnol Bioeng. 2006;95(1):1–7.
119. Koike N, Fukumura D, Gralla O, Au P, Schechner JS, Jain RK. Tissue engineering: creation of long-lasting blood vessels. Nature. 2004;428(6979):138–9.
120. le Noble F, Moyon D, Pardanaud L, Yuan L, Djonov V, Matthijsen R, Breant C, Fleury V, Eichmann A. Flow regulates arterial-venous differentiation in the chick embryo yolk sac. Development. 2004;131(2):361–75.
121. van der Meer A, Vermeul K, Poot AA, Feijen J, Vermes I. A microfluidic wound healing assay for quantifying endothelial cell migration. Am J Physiol Heart Circ Physiol. 2010;298(2):H719–25.
122. Metaxa E, Meng H, Kaluvala SR, Szymanski MP, Paluch RA, Kolega J. Nitric oxide-dependent stimulation of endothelial cell proliferation by sustained high flow. Am J Physiol Heart Circ Physiol. 2008;295(2):H736–42.
123. Punchard MA, Stenson-Cox C, O'Cearbhaill E D, Lyons E, Gundy S, Murphy L, Pandit A, McHugh PE, Barron V. Endothelial cell response to biomechanical forces under simulated vascular loading conditions. J Biomech. 2007;40(14):3146–54.
124. Hoepken S, Fuhrmann R, Jung F, Franke RP. Shear resistance of human umbilical endothelial cells on different materials covered with or without extracellular matrix: controlled in-vitro study. Clin Hemorheol Microcirc. 2009;43(1):157–66.
125. Rabbany SY, Yamamoto M, James D, Li H, Butler J, Rafii S. Generation of stable co-cultures of vascular cells in a honeycomb alginate scaffold. Tissue Eng Part A. 2010;16(1):299–308.
126. Kang H, Bayless KJ, Kaunas R. Fluid shear stress modulates endothelial cell invasion into three-dimensional collagen matrices. Am J Physiol Heart Circ Physiol. 2008;295(5):H2087–97.
127. Ueda A, Koga M, Ikeda M, Kudo S, Tanishita K. Effect of shear stress on microvessel network formation of endothelial cells with *in vitro* three-dimensional model. Am J Physiol Heart Circ Physiol. 2004;287(3):H994–1002.
128. Helm CL, Zisch A, Swartz MA. Engineered blood and lymphatic capillaries in 3-D VEGF-fibrin-collagen matrices with interstitial flow. Biotechnol Bioeng. 2007;96(1):167–76.
129. Ng CP, Swartz MA. Mechanisms of interstitial flow-induced remodeling of fibroblast-collagen cultures. Ann Biomed Eng. 2006;34(3):446–54.
130. Yoon SK, Ahn YH, Jeong MH. Effect of culture temperature on follicle-stimulating hormone production by Chinese hamster ovary cells in a perfusion bioreactor. Appl Microbiol Biotechnol. 2007;76(1):83–9.
131. Cho SW, Song KW, Rhie JW, Park MH, Choi CY, Kim BS. Engineered adipose tissue formation enhanced by basic fibroblast growth factor and a mechanically stable environment. Cell Transplant. 2007;16(4):421–34.
132. Timmins NE, Scherberich A, Fruh JA, Heberer M, Martin I, Jakob M. Three-dimensional cell culture and tissue engineering in a T-CUP (tissue culture under perfusion). Tissue Eng. 2007;13(8):2021–8.
133. De Bartolo L, Piscioneri A, Cotroneo G, Salerno S, Tasselli F, Campana C, Morelli S, Rende M, Caroleo MC, Bossio M, Drioli E. Human lymphocyte PEEK-WC hollow fiber membrane bioreactor. J Biotechnol. 2007;132(1):65–74.
134. Ye H, Xia Z, Ferguson DJ, Triffitt JT, Cui Z. Studies on the use of hollow fibre membrane bioreactors for tissue generation by using rat bone marrow fibroblastic cells and a composite scaffold. J Mater Sci Mater Med. 2007;18(4):641–8.
135. Dahl SL, Koh J, Prabhakar V, Niklason LE. Decellularized native and engineered arterial scaffolds for transplantation. Cell Transplant. 2003;12(6):659–66.
136. McFetridge PS, Abe K, Horrocks M, Chaudhuri JB. Vascular tissue engineering: bioreactor design considerations for extended culture of primary human vascular smooth muscle cells. ASAIO J. 2007;53(5):623–30.
137. Teebken OE, Puschmann C, Breitenbach I, Rohde B, Burgwitz K, Haverich A. Preclinical development of tissue-engineered vein valves and venous substitutes using re-endothelialised human vein matrix. Eur J Vasc Endovasc Surg. 2009;37(1):92–102.
138. Aper T, Schmidt A, Duchrow M, Bruch HP. Autologous blood vessels engineered from peripheral blood sample. Eur J Vasc Endovasc Surg. 2007;33(1):33–9.
139. Aper T, Haverich A, Teebken O. New developments in tissue engineering of vascular prosthetic grafts. Vasa. 2009;38(2):99–122.
140. Cardinal KO, Bonnema GT, Hofer H, Barton JK, Williams SK. Tissue-engineered vascular grafts as *in vitro* blood vessel mimics for the evaluation of endothelialization of intravascular devices. Tissue Eng. 2006;12(12):3431–8.
141. Chrobak KM, Potter DR, Tien J. Formation of perfused, functional microvascular tubes *in vitro*. Microvasc Res. 2006;71(3):185–96.
142. Teebken OE, Haverich A. Tissue engineering of small diameter vascular grafts. Eur J Vasc Endovasc Surg. 2002;23(6):475–85.
143. Chang CC, Nunes SS, Sibole SC, Krishnan L, Williams SK, Weiss JA, Hoying JB. Angiogenesis in a microvascular construct for transplantation depends on the method of chamber circulation. Tissue Eng Part A. 2010;16(3):795–805.

144. Chiu LL, Montgomery M, Liang Y, Liu H, Radisic M. Perfusable branching microvessel bed for vascularization of engineered tissues. Proc Natl Acad Sci U S A. 2012;109(50):E3414–23.
145. Chan JM, Zervantonakis IK, Rimchala T, Polacheck WJ, Whisler J, Kamm RD. Engineering of *in vitro* 3D capillary beds by self-directed angiogenic sprouting. PLoS One. 2012;7(12):e50582.
146. Tanihara M, Suzuki Y, Yamamoto E, Noguchi A, Mizushima Y. Sustained release of basic fibroblast growth factor and angiogenesis in a novel covalently crosslinked gel of heparin and alginate. J Biomed Mater Res. 2001;56(2):216–21.
147. Wenk E, Murphy AR, Kaplan DL, Meinel L, Merkle HP, Uebersax L. The use of sulfonated silk fibroin derivatives to control binding, delivery and potency of FGF-2 in tissue regeneration. Biomaterials. 2010;31(6):1403–13.
148. Takayama T, Taguchi T, Koyama H, Sakari M, Kamimura W, Takato T, Miyata T, Nagawa H. The growth of a vascular network inside a collagen-citric acid derivative hydrogel in rats. Biomaterials. 2009;30(21):3580–7.
149. Gomez G, Korkiakoski S, Gonzalez MM, Lansman S, Ella V, Salo T, Kellomaki M, Ashammakhi N, Arnaud E. Effect of FGF and polylactide scaffolds on calvarial bone healing with growth factor on biodegradable polymer scaffolds. J Craniofac Surg. 2006;17(5):935–42.
150. Inoue S, Kijima H, Kidokoro M, Tanaka M, Suzuki Y, Motojuku M, Inokuchi S. The effectiveness of basic fibroblast growth factor in fibrin-based cultured skin substitute *in vivo*. J Burn Care Res. 2009;30(3):514–9.
151. Takaoka R, Hikasa Y, Tabata Y. Vascularization around poly(tetrafluoroethylene) mesh with coating of gelatin hydrogel incorporating basic fibroblast growth factor. J Biomater Sci Polym Ed. 2009;20(10):1483–94.
152. Strieth S, Weger T, Bartesch C, Bittmann P, Stelter K, Berghaus A. Biocompatibility of porous polyethylene implants tissue-engineered by extracellular matrix and VEGF. J Biomed Mater Res A. 2010;93(4):1566–73.
153. Chen RR, Silva EA, Yuen WW, Mooney DJ. Spatio-temporal VEGF and PDGF delivery patterns blood vessel formation and maturation. Pharm Res. 2007;24(2):258–64.
154. Rocha FG, Sundback CA, Krebs NJ, Leach JK, Mooney DJ, Ashley SW, Vacanti JP, Whang EE. The effect of sustained delivery of vascular endothelial growth factor on angiogenesis in tissue-engineered intestine. Biomaterials. 2008;29(19):2884–90.
155. Gafni Y, Zilberman Y, Ophir Z, Abramovitch R, Jaffe M, Gazit Z, Domb A, Gazit D. Design of a filamentous polymeric scaffold for *in vivo* guided angiogenesis. Tissue Eng. 2006;12(11):3021–34.
156. Ford MC, Bertram JP, Hynes SR, Michaud M, Li Q, Young M, Segal SS, Madri JA, Lavik EB. A macroporous hydrogel for the coculture of neural progenitor and endothelial cells to form functional vascular networks *in vivo*. Proc Natl Acad Sci U S A. 2006;103(8):2512–7.
157. Kellar RS, Landeen LK, Shepherd BR, Naughton GK, Ratcliffe A, Williams SK. Scaffold-based three-dimensional human fibroblast culture provides a structural matrix that supports angiogenesis in infarcted heart tissue. Circulation. 2001;104(17):2063–8.
158. Schechner JS, Nath AK, Zheng L, Kluger MS, Hughes CC, Sierra-Honigmann MR, Lorber MI, Tellides G, Kashgarian M, Bothwell AL, Pober JS. *In vivo* formation of complex microvessels lined by human endothelial cells in an immunodeficient mouse. Proc Natl Acad Sci U S A. 2000;97(16):9191–6.
159. Messina A, Bortolotto SK, Cassell OC, Kelly J, Abberton KM, Morrison WA. Generation of a vascularized organoid using skeletal muscle as the inductive source. FASEB J. 2005;19(11):1570–2.
160. Flynn L, Semple JL, Woodhouse KA. Decellularized placental matrices for adipose tissue engineering. J Biomed Mater Res A. 2006;79(2):359–69.
161. Schultheiss D, Gabouev AI, Cebotari S, Tudorache I, Walles T, Schlote N, Wefer J, Kaufmann PM, Haverich A, Jonas U, Stief CG, Mertsching H. Biological vascularized matrix for bladder tissue engineering: matrix preparation, reseeding technique and short-term implantation in a porcine model. J Urol. 2005;173(1):276–80.
162. Watzka SB, Steiner M, Samorapoompichit P, Gross K, Coles JG, Wolner E, Weigel G. Establishment of vessel-like structures in long-term three-dimensional tissue culture of myocardium: an electron microscopy study. Tissue Eng. 2004;10(11–12):1684–94.
163. Yu DY, Su EN, Cringle SJ, Yu PK. Isolated preparations of ocular vasculature and their applications in ophthalmic research. Prog Retin Eye Res. 2003;22(2):135–69.
164. Ucuzian AA, Greisler HP. *In vitro* models of angiogenesis. World J Surg. 2007;31(4):654–63.
165. Folkman J. Angiogenesis: initiation and control. Ann N Y Acad Sci. 1982;401:212–27.
166. Gamble JR, Matthias LJ, Meyer G, Kaur P, Russ G, Faull R, Berndt MC, Vadas MA. Regulation of *in vitro* capillary tube formation by anti-integrin antibodies. J Cell Biol. 1993;121(4):931–43.
167. Sholley MM, Ferguson GP, Seibel HR, Montour JL, Wilson JD. Mechanisms of neovascularization. Vascular sprouting can occur without proliferation of endothelial cells. Lab Invest. 1984;51(6):624–34.
168. Montanez E, Casaroli-Marano RP, Vilaro S, Pagan R. Comparative study of tube assembly in three-dimensional collagen matrix and on Matrigel coats. Angiogenesis. 2002;5(3):167–72.
169. Yamamura N, Sudo R, Ikeda M, Tanishita K. Effects of the mechanical properties of collagen gel on the *in vitro* formation of microvessel networks by endothelial cells. Tissue Eng. 2007;13(7):1443–53.
170. Ehrmann RL, Gey GO. The growth of cells on a transparent gel of reconstituted rat-tail collagen. J Natl Cancer Inst. 1956;16(6):1375–403.

171. Sibinga NE, Foster LC, Hsieh CM, Perrella MA, Lee WS, Endege WO, Sage EH, Lee ME, Haber E. Collagen VIII is expressed by vascular smooth muscle cells in response to vascular injury. Circ Res. 1997;80(4):532–41.
172. Sutmuller M, Bruijn JA, de Heer E. Collagen types VIII and X, two non-fibrillar, short-chain collagens. Structure homologies, functions and involvement in pathology. Histol Histopathol. 1997;12(2):557–66.
173. de Lange Davies C, Melder JR, Munn LL, Mouta-Carreira C, Jain KR, Boucher Y. Decorin inhibits endothelial migration and tube-like structure formation: Role of thrombospondin-1. Microvasc Res. 2001;62:26–42.
174. Schonherr E, Levkau B, Schaefer L, Kresse H, Walsh K. Decorin affects endothelial cells by Akt-dependent and -independent pathways. Ann N Y Acad Sci. 2002;973(1):149–52.
175. Jarvelainen HT, Iruela-Arispe ML, Kinsella MG, Sandell LJ, Sage EH, Wight TN. Expression of decorin by sprouting bovine aortic endothelial cells exhibiting angiogenesis *in vitro*. Exp Cell Res. 1992;203(2):395–401.
176. Zagzag D, Shiff B, Jallo GI, Greco MA, Blanco C, Cohen H, Hukin J, Allen JC, Friedlander DR. Tenascin-C promotes microvascular cell migration and phosphorylation of focal adhesion kinase. Cancer Res. 2002;62(9):2660–8.
177. Kim CH, Bak KH, Kim YS, Kim JM, Ko Y, Oh SJ, Kim KM, Hong EK. Expression of tenascin-C in astrocytic tumors: its relevance to proliferation and angiogenesis. Surg Neurol. 2000;54(3):235–40.
178. Stupack GD, Cheresh AD. ECM remodeling regulates angiogenesis: endothelial integrins look for new ligands. Sci STKE. 2002;2002(119):pe7.
179. Malan D, Wenzel D, Schmidt A, Geisen C, Raible A, Bolck B, Fleischmann BK, Bloch W. Endothelial beta1 integrins regulate sprouting and network formation during vascular development. Development. 2010;137(6):993–1002.
180. Yano Y, Geibel J, Sumpio BE. Cyclic strain induces reorganization of integrin alpha 5 beta 1 and alpha 2 beta 1 in human umbilical vein endothelial cells. J Cell Biochem. 1997;64(3):505–13.
181. Montgomery AM, Reisfeld RA, Cheresh DA. Integrin alpha v beta 3 rescues melanoma cells from apoptosis in three-dimensional dermal collagen. Proc Natl Acad Sci USA. 1994;91(19):8856–60.
182. Vailhe B, Ronot X, Tracqui P, Usson Y, Tranqui L. *In vitro* angiogenesis is modulated by the mechanical properties of fibrin gels and is related to alpha(v) beta3 integrin localization. *In Vitro* Cell Dev Biol Anim. 1997;33(10):763–73.
183. Eliceiri BP, Cheresh DA. The role of alphav integrins during angiogenesis: insights into potential mechanisms of action and clinical development. J Clin Invest. 1999;103(9):1227–30.
184. Ghosh K, Pan Z, Guan E, Ge S, Liu Y, Nakamura T, Ren XD, Rafailovich M, Clark RA. Cell adaptation to a physiologically relevant ECM mimic with different viscoelastic properties. Biomaterials. 2007;28(4):671–9.
185. Hsu S, Thakar R, Liepmann D, Li S. Effects of shear stress on endothelial cell haptotaxis on micropatterned surfaces. Biochem Biophys Res Commun. 2005;337(1):401–9.
186. Lo CM, Wang HB, Dembo M, Wang YL. Cell movement is guided by the rigidity of the substrate. Biophys J. 2000;79(1):144–52.
187. Pelham RJ Jr, Wang Y. Cell locomotion and focal adhesions are regulated by substrate flexibility. Proc Natl Acad Sci U S A. 1997;94(25):13661–5.
188. Streuli CH, Bissell MJ. Expression of extracellular matrix components is regulated by substratum. J Cell Biol. 1990;110(4):1405–15.
189. Pelham RJ Jr, Wang Y. High resolution detection of mechanical forces exerted by locomoting fibroblasts on the substrate. Mol Biol Cell. 1999;10(4):935–45.
190. Ehrlich HP, Rajaratnam JB. Cell locomotion forces versus cell contraction forces for collagen lattice contraction: an *in vitro* model of wound contraction. Tissue Cell. 1990;22(4):407–17.
191. Roeder BA, Kokini K, Waisner BZ, Sturgis JE, Robinson JP, Voytik-Harbin SL. Extracellular matrix (ECM) microstructure determines ECM-Cell strain transfer in 3D tissue constructus. ASME Summer Bioengineering Conference; 2003 June 25–29; Key Biscayne, FL. Available at: http://www.tulane.edu/~sbc2003/pdfdocs/0301.PDF
192. Sheetz MP, Felsenfeld DP, Galbraith CG. Cell migration: regulation of force on extracellular-matrix-integrin complexes. Trends Cell Biol. 1998;8(2):51–4.
193. Parker KK, Brock AL, Brangwynne C, Mannix RJ, Wang N, Ostuni E, Geisse NA, Adams JC, Whitesides GM, Ingber DE. Directional control of lamellipodia extension by constraining cell shape and orienting cell tractional forces. FASEB J. 2002;16(10):1195–204.
194. Choquet D, Felsenfeld DP, Sheetz MP. Extracellular matrix rigidity causes strengthening of integrin-cytoskeleton linkages. Cell. 1997;88(1):39–48.
195. Uttayarat P, Toworfe GK, Dietrich F, Lelkes PI, Composto RJ. Topographic guidance of endothelial cells on silicone surfaces with micro- to nanogrooves: orientation of actin filaments and focal adhesions. J Biomed Mater Res A. 2005;75(3):668–80.
196. Ingber D. Tensegrity: the architectural basis of cellular mechanotransduction. Annu Rev Physiol. 1997;59:575–99.
197. Deroanne CF, Lapiere CM, Nusgens BV. *In vitro* tubulogenesis of endothelial cells by relaxation of the coupling extracellular matrix-cytoskeleton. Cardiovasc Res. 2001;49(3):647–58.
198. Sieminski AL, Hebbel RP, Gooch KJ. The relative magnitudes of endothelial force generation and matrix stiffness modulate capillary morphogenesis *in vitro*. Exp Cell Res. 2004;297(2):574–84.
199. Krishnan L, Hoying JB, Nguyen H, Song H, Weiss JA. Interaction of angiogenic microvessels with the extracellular matrix. Am J Physiol Heart Circ Physiol. 2007;293(6):H3650–8.

200. Taraboletti G, D'Ascenzo S, Borsotti P, Giavazzi R, Pavan A, Dolo V. Shedding of the matrix metalloproteinases MMP-2, MMP-9, and MT1-MMP as membrane vesicle-associated components by endothelial cells. Am J Pathol. 20022002;160(2):673–80.
201. Zhu W-H, Guo X, Villaschi S, Nicosia RF. Regulation of vascular growth and regression by matrix metalloproteinases in the rat aorta model of angiogenesis. Lab Invest. 2000;80(4):545–55.
202. Ghajar CM, Chen X, Harris JW, Suresh V, Hughes CC, Jeon NL, Putnam AJ, George SC. The effect of matrix density on the regulation of 3-D capillary morphogenesis. Biophys J. 2008;94(5):1930–41.
203. Ghajar CM, Blevins KS, Hughes CC, George SC, Putnam AJ. Mesenchymal stem cells enhance angiogenesis in mechanically viable prevascularized tissues via early matrix metalloproteinase upregulation. Tissue Eng. 2006;12(10):2875–88.
204. Anand-Apte B, Pepper MS, Voest E, Montesano R, Olsen B, Murphy G, Apte SS, Zetter B. Inhibition of angiogenesis by tissue inhibitor of metalloproteinase-3. Invest Ophthalmol Vis Sci. 1997;38(5):817–23.
205. Ray JM, Stetler-Stevenson WG. The role of matrix metalloproteases and their inhibitors in tumour invasion, metastasis and angiogenesis. Eur Respir J. 1994;7(11):2062–72.
206. Clowes AW, Kirkman TR, Reidy MA. Mechanisms of arterial graft healing. Rapid transmural capillary ingrowth provides a source of intimal endothelium and smooth muscle in porous PTFE prostheses. Am J Pathol. 1986;123(2):220–30.
207. Clowes AW, Zacharias RK, Kirkman TR. Early endothelial coverage of synthetic arterial grafts: porosity revisited. Am J Surg. 1987;153(5):501–4.
208. Sharkawy AA, Klitzman B, Truskey GA, Reichert WM. Engineering the tissue which encapsulates subcutaneous implants. II. Plasma-tissue exchange properties. J Biomed Mater Res. 1998;40(4):586–97.
209. Padera RF, Colton CK. Time course of membrane microarchitecture-driven neovascularization. Biomaterials. 1996;17(3):277–84.
210. Siefert SA, Sarkar R. Matrix metalloproteinases in vascular physiology and disease. Vascular. 2012;20(4):210–6.
211. Sage EH, Vernon RB. Regulation of angiogenesis by extracellular matrix: the growth and the glue. J Hypertens Suppl. 1994;12(10):S145–52.
212. Vernon RB, Angello JC, Iruela-Arispe ML, Lane TF, Sage EH. Reorganization of basement membrane matrices by cellular traction promotes the formation of cellular networks *in vitro*. Lab Invest. 1992;66(5):536–47.
213. Stopak D, Harris AK. Connective tissue morphogenesis by fibroblast traction. I. Tissue culture observations. Dev Biol. 1982;90(2):383–98.
214. Grinnell F, Lamke CR. Reorganization of hydrated collagen lattices by human skin fibroblasts. J Cell Sci. 1984;66:51–63.
215. Vernon R, Sage E. Contraction of fibrillar type I collagen by endothelial cells: a study *in vitro*. J Cell Biochem. 1996;60:185–97.
216. Wolf K, Wu YI, Liu Y, Geiger J, Tam E, Overall C, Stack MS, Friedl P. Multi-step pericellular proteolysis controls the transition from individual to collective cancer cell invasion. Nat Cell Biol. 2007;9(8):893–904.
217. Brooks PC, Stromblad S, Sanders LC, von Schalscha TL, Aimes RT, Stetler-Stevenson WG, Quigley JP, Cheresh DA. Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin alpha v beta 3. Cell. 1996;85(5):683–93.
218. Ruberti JW, Hallab NJ. Strain-controlled enzymatic cleavage of collagen in loaded matrix. Biochemical and biophysical research communications. 2005;336(2):483–9.
219. Patel N, Padera R, Sanders GH, Cannizzaro SM, Davies MC, Langer R, Roberts CJ, Tendler SJ, Williams PM, Shakesheff KM. Spatially controlled cell engineering on biodegradable polymer surfaces. FASEB J. 1998;12(14):1447–54.
220. Hsieh P, Chen LB. Behavior of cells seeded in isolated fibronectin matrices. J Cell Biol. 1983;96(5):1208–17.
221. Mrksich M, Dike LE, Tien J, Ingber DE, Whitesides GM. Using microcontact printing to pattern the attachment of mammalian cells to self-assembled monolayers of alkanethiolates on transparent films of gold and silver. Exp Cell Res. 1997;235(2):305–13.
222. Uttayarat P, Chen M, Li M, Allen FD, Composto RJ, Lelkes PI. Microtopography and flow modulate the direction of endothelial cell migration. Am J Physiol Heart Circ Physiol. 2008;294(2):H1027–35.
223. Rajnicek A, McCaig C. Guidance of CNS growth cones by substratum grooves and ridges: effects of inhibitors of the cytoskeleton, calcium channels and signal transduction pathways. J Cell Sci. 1997;110(Pt 23):2915–24.
224. Wang JH, Jia F, Gilbert TW, Woo SL. Cell orientation determines the alignment of cell-produced collagenous matrix. J Biomech. 2003;36(1):97–102.
225. Koh HS, Yong T, Teo WE, Chan CK, Puhaindran ME, Tan TC, Lim A, Lim BH, Ramakrishna S. *In vivo* study of novel nanofibrous intra-luminal guidance channels to promote nerve regeneration. J Neural Eng. 2010;7(4):046003.
226. Hadjizadeh A, Doillon CJ, Vermette P. Bioactive polymer fibers to direct endothelial cell growth in a three-dimensional environment. Biomacromolecules. 2007;8(3):864–73.
227. Nagata I, Kawana A, Nakatsuji N. Perpendicular contact guidance of CNS neuroblasts on artificial microstructures. Development. 1993;117(1):401–8.
228. Matsuda T, Sugawara T. Control of cell adhesion, migration, and orientation on photochemically microprocessed surfaces. J Biomed Mater Res. 1996;32(2):165–73.
229. Clark P, Connolly P, Moores GR. Cell guidance by micropatterned adhesiveness *in vitro*. J Cell Sci. 1992;103(Pt 1):287–92.

230. Teixeira AI, Abrams GA, Bertics PJ, Murphy CJ, Nealey PF. Epithelial contact guidance on well-defined micro- and nanostructured substrates. J Cell Sci. 2003;116(Pt 10):1881–92.
231. Harris AK, Stopak D, Wild P. Fibroblast traction as a mechanism for collagen morphogenesis. Nature. 1981;290(5803):249–51.
232. Wu P, Hoying JB, Williams SK, Kozikowski BA, Lauffenburger DA. Integrin-binding peptide in solution inhibits or enhances endothelial cell migration, predictably from cell adhesion. Ann Biomed Eng. 1994;22(2):144–52.
233. Hoying JB, Williams SK. Effects of basic fibroblast growth factor on human microvessel endothelial cell migration on collagen I correlates inversely with adhesion and is cell density dependent. J Cell Physiol. 1996;168(2):294–304.
234. Sazonova OV, Lee KL, Isenberg BC, Rich CB, Nugent MA, Wong JY. Cell-cell interactions mediate the response of vascular smooth muscle cells to substrate stiffness. Biophys J. 2011;101(3):622–30.
235. Stroka KM, Aranda-Espinoza H. Effects of morphology vs. cell-cell interactions on endothelial cell stiffness. Cell Mol Bioeng. 2011;4(1):9–27.
236. Chicurel ME, Chen CS, Ingber DE. Cellular control lies in the balance of forces. Curr Opin Cell Biol. 1998;10(2):232–9.
237. Lee PF, Yeh AT, Bayless KJ. Nonlinear optical microscopy reveals invading endothelial cells anisotropically alter three-dimensional collagen matrices. Exp Cell Res. 2009;315(3):396–410.
238. Edgar LT, Sibole SC, Underwood CJ, Guilkey JE, Weiss JA. A computational model of *in vitro* angiogenesis based on extracellular matrix fibre orientation. Comput Methods Biomech Biomed Engin. 2013;16(7):790–801.
239. Rivilis I, Milkiewicz M, Boyd P, Goldstein J, Brown MD, Egginton S, Hansen FM, Hudlicka O, Haas TL. Differential involvement of MMP-2 and VEGF during muscle stretch- versus shear stress-induced angiogenesis. Am J Physiol Heart Circ Physiol. 2002;283(4):H1430–8.
240. Egginton S, Zhou AL, Brown MD, Hudlicka O. Unorthodox angiogenesis in skeletal muscle. Cardiovasc Res. 2001;49(3):634–46.
241. Prior BM, Yang HT, Terjung RL. What makes vessels grow with exercise training? J Appl Physiol. 2004;97(3):1119–28.
242. Pries AR, Cornelissen AJ, Sloot AA, Hinkeldey M, Dreher MR, Hopfner M, Dewhirst MW, Secomb TW. Structural adaptation and heterogeneity of normal and tumor microvascular networks. PLoS Comput Biol. 2009;5(5):e1000394.
243. Zhao S, Suciu A, Ziegler T, Moore JE Jr, Burki E, Meister JJ, Brunner HR. Synergistic effects of fluid shear stress and cyclic circumferential stretch on vascular endothelial cell morphology and cytoskeleton. Arterioscler Thromb Vasc Biol. 1995;15(10):1781–6.
244. Joung IS, Iwamoto MN, Shiu YT, Quam CT. Cyclic strain modulates tubulogenesis of endothelial cells in a 3D tissue culture model. Microvasc Res. 2006;71(1):1–11.
245. Shirinsky VP, Antonov AS, Birukov KG, Sobolevsky AV, Romanov YA, Kabaeva NV, Antonova GN, Smirnov VN. Mechano-chemical control of human endothelium orientation and size. J Cell Biol. 1989;109(1):331–9.
246. Ives CL, Eskin SG, McIntire LV. Mechanical effects on endothelial cell morphology: *in vitro* assessment. *In Vitro* Cell Dev Biol. 1986;22(9):500–7.
247. Wang JH, Goldschmidt-Clermont P, Wille J, Yin FC. Specificity of endothelial cell reorientation in response to cyclic mechanical stretching. J Biomech. 2001;34(12):1563–72.
248. Moretti M, Prina-Mello A, Reid AJ, Barron V, Prendergast PJ. Endothelial cell alignment on cyclically-stretched silicone surfaces. J Mater Sci Mater Med. 2004;15(10):1159–64.
249. Neidlinger-Wilke C, Grood E, Claes L, Brand R. Fibroblast orientation to stretch begins within three hours. J Orthop Res. 2002;20(5):953–6.
250. Barocas VH, Tranquillo RT. An anisotropic biphasic theory of tissue-equivalent mechanics: the interplay among cell traction, fibrillar network deformation, fibril alignment, and cell contact guidance. J Biomech Eng. 1997;119(2):137–45.
251. Lopez Valle CA, Auger FA, Rompre P, Bouvard V, Germain L. Peripheral anchorage of dermal equivalents. Br J Dermatol. 1992;127(4):365–71.
252. L'Heureux N, Germain L, Labbe R, Auger FA. *In vitro* construction of a human blood vessel from cultured vascular cells: a morphologic study. J Vasc Surg. 1993;17(3):499–509.
253. Wang H, Ip W, Boissy R, Grood ES. Cell orientation response to cyclically deformed substrates: experimental validation of a cell model. J Biomech. 1995;28(12):1543–52.
254. Buck RC. Reorientation response of cells to repeated stretch and recoil of the substratum. Exp Cell Res. 1980;127(2):470–4.
255. Buckley MJ, Banes AJ, Levin LG, Sumpio BE, Sato M, Jordan R, Gilbert J, Link GW, Tran Son Tay R. Osteoblasts increase their rate of division and align in response to cyclic, mechanical tension *in vitro*. Bone Miner. 1988;4(3):225–36.
256. Dartsch PC, Hammerle H. Orientation response of arterial smooth muscle cells to mechanical stimulation. Eur J Cell Biol. 1986;41(2):339–46.
257. Takemasa T, Sugimoto K, Yamashita K. Amplitude-dependent stress fiber reorientation in early response to cyclic strain. Exp Cell Res. 1997;230(2):407–10.
258. Wille JJ, Ambrosi CM, Yin FC. Comparison of the effects of cyclic stretching and compression on endothelial cell morphological responses. J Biomech Eng. 2004;126(5):545–51.
259. Katanosaka Y, Bao JH, Komatsu T, Suemori T, Yamada A, Mohri S, Naruse K. Analysis of cyclic-stretching responses using cell-adhesion-patterned cells. J Biotechnol. 2008;133(1):82–9.

260. Burridge K, Fath K, Kelly T, Nuckolls G, Turner C. Focal adhesions: transmembrane junctions between the extracellular matrix and the cytoskeleton. Annu Rev Cell Biol. 1988;4:487–525.
261. Neidlinger-Wilke C, Grood ES, Wang J-C, Brand RA, Claes L. Cell alignment is induced by cyclic changes in cell length: studies of cells grown in cyclically stretched substrates. J Orthop Res. 2001;19(2):286–93.
262. Eastwood M, Mudera VC, McGrouther DA, Brown RA. Effect of precise mechanical loading on fibroblast populated collagen lattices: morphological changes. Cell Motil Cytoskeleton. 1998;40(1):13–21.
263. Takemasa T, Yamaguchi T, Yamamoto Y, Sugimoto K, Yamashita K. Oblique alignment of stress fibers in cells reduces the mechanical stress in cyclically deforming fields. Eur J Cell Biol. 1998;77(2):91–9.
264. Hannafin JA, Arnoczky SP, Hoonjan A, Torzilli PA. Effect of stress deprivation and cyclic tensile loading on the material and morphologic properties of canine flexor digitorum profundus tendon: an *in vitro* study. J Orthop Res. 1995;13(6):907–14.
265. Majima T, Marchuk LL, Shrive NG, Frank CB, Hart DA. In-vitro cyclic tensile loading of an immobilized and mobilized ligament autograft selectively inhibits mRNA levels for collagenase (MMP-1). J Orthop Sci. 2000;5(5):503–10.
266. Arnoczky SP, Tian T, Lavagnino M, Gardner K. *Ex vivo* static tensile loading inhibits MMP-1 expression in rat tail tendon cells through a cytoskeletally based mechanotransduction mechanism. J Orthop Res. 2004;22(2):328–33.
267. Williams B. Mechanical influences on vascular smooth muscle cell function. J Hypertens. 1998;16(12 Pt 2):1921–9.
268. Ingber DE, Folkman J. How does extracellular matrix control capillary morphogenesis? Cell. 1989;58(5):803–5.
269. Eastwood M, Porter R, Khan U, McGrouther G, Brown R. Quantitative analysis of collagen gel contractile forces generated by dermal fibroblasts and the relationship to cell morphology. J Cell Physiol. 1996;166(1):33–42.
270. Brown RA, Wiseman M, Chuo CB, Cheema U, Nazhat SN. Ultrarapid engineering of biomimetic materials and tissues: fabrication of nano- and microstructures by plastic compression. Adv Funct Mater. 2005;15(11):1762–70.
271. Haston WS, Shields JM, Wilkinson PC. The orientation of fibroblasts and neutrophils on elastic substrata. Exp Cell Res. 1983;146(1):117–26.
272. Davis GE, Camarillo CW. Regulation of endothelial cell morphogenesis by integrins, mechanical forces, and matrix guidance pathways. Exp Cell Res. 1995;216(1):113–23.
273. Eastwood M, McGrouther DA, Brown RA. A culture force monitor for measurement of contraction forces generated in human dermal fibroblast cultures: evidence for cell-matrix mechanical signalling. Biochim Biophys Acta. 1994;1201(2):186–92.
274. Takakuda K, Miyairi H. Tensile behaviour of fibroblasts cultured in collagen gel. Biomaterials. 1996;17(14):1393–7.
275. Brown RA, Prajapati R, McGrouther DA, Yannas IV, Eastwood M. Tensional homeostasis in dermal fibroblasts: mechanical responses to mechanical loading in three-dimensional substrates. J Cell Physiol. 1998;175(3):323–32.
276. Guido S, Tranquillo RT. A methodology for the systematic and quantitative study of cell contact guidance in oriented collagen gels. Correlation of fibroblast orientation and gel birefringence. J Cell Sci. 1993;105(Pt 2):317–31.
277. Mudera VC, Pleass R, Eastwood M, Tarnuzzer R, Schultz G, Khaw P, McGrouther DA, Brown RA. Molecular responses of human dermal fibroblasts to dual cues: contact guidance and mechanical load. Cell Motil Cytoskeleton. 2000;45(1):1–9.
278. Loesberg WA, Walboomers XF, van Loon JJ, Jansen JA. The effect of combined cyclic mechanical stretching and microgrooved surface topography on the behavior of fibroblasts. J Biomed Mater Res A. 2005;75(3):723–32.
279. Li S, Butler P, Wang Y, Hu Y, Han DC, Usami S, Guan JL, Chien S. The role of the dynamics of focal adhesion kinase in the mechanotaxis of endothelial cells. Proc Natl Acad Sci U S A. 2002;99(6):3546–51.
280. Chiu JJ, Chien S. Effects of disturbed flow on vascular endothelium: pathophysiological basis and clinical perspectives. Physiol Rev. 2011;91(1):327–87.
281. Chien S. Mechanotransduction and endothelial cell homeostasis: the wisdom of the cell. Am J Physiol Heart Circ Physiol. 2007;292(3):H1209–24.
282. Chien S. Effects of disturbed flow on endothelial cells. Ann Biomed Eng. 2008;36(4):554–62.
283. Pyke KE, Tschakovsky ME. The relationship between shear stress and flow-mediated dilatation: implications for the assessment of endothelial function. J Physiol. 2005;568(Pt 2):357–69.
284. Ookawa K, Sato M, Ohshima N. Morphological changes of endothelial cells after exposure to fluid-imposed shear stress: differential responses induced by extracellular matrices. Biorheology. 1993;30(2):131–40.
285. Thoumine O, Nerem RM, Girard PR. Changes in organization and composition of the extracellular matrix underlying cultured endothelial cells exposed to laminar steady shear stress. Lab Invest. 1995;73(4):565–76.
286. Ookawa K, Sato M, Ohshima N. Changes in the microstructure of cultured porcine aortic endothelial cells in the early stage after applying a fluid-imposed shear stress. J Biomech. 1992;25(11):1321–8.
287. McCue S, Dajnowiec D, Xu F, Zhang M, Jackson MR, Langille BL. Shear stress regulates forward and reverse planar cell polarity of vascular endothelium *in vivo* and *in vitro*. Circ Res. 2006;98(7):939–46.
288. Davies PF. Flow-mediated endothelial mechanotransduction. Physiol Rev. 1995;75(3):519–60.
289. Ng CP, Hinz B, Swartz MA. Interstitial fluid flow induces myofibroblast differentiation and collagen alignment *in vitro*. J Cell Sci. 2005;118(Pt 20):4731–9.

290. Ng CP, Helm CL, Swartz MA. Interstitial flow differentially stimulates blood and lymphatic endothelial cell morphogenesis *in vitro*. Microvasc Res. 2004;68(3):258–64.
291. Kane RS, Takayama S, Ostuni E, Ingber DE, Whitesides GM. Patterning proteins and cells using soft lithography. Biomaterials. 1999;20(23–24):2363–76.
292. Nakatsu MN, Sainson RC, Aoto JN, Taylor KL, Aitkenhead M, Perez-del-Pulgar S, Carpenter PM, Hughes CC. Angiogenic sprouting and capillary lumen formation modeled by human umbilical vein endothelial cells (HUVEC) in fibrin gels: the role of fibroblasts and Angiopoietin-1. Microvasc Res. 2003;66(2):102–12.
293. Nicosia RF, Lin YJ, Hazelton D, Qian X. Endogenous regulation of angiogenesis in the rat aorta model. Role of vascular endothelial growth factor. Am J Pathol. 1997;151(5):1379–86.
294. Tille JC, Pepper MS. Mesenchymal cells potentiate vascular endothelial growth factor-induced angiogenesis *in vitro*. Exp Cell Res. 2002;280(2):179–91.
295. Hidaka C, Ibarra C, Hannafin JA, Torzilli PA, Quitoriano M, Jen SS, Warren RF, Crystal RG. Formation of vascularized meniscal tissue by combining gene therapy with tissue engineering. Tissue Eng. 2002;8(1):93–105.
296. Yu J, Lei L, Liang Y, Hinh L, Hickey RP, Huang Y, Liu D, Yeh JL, Rebar E, Case C, Spratt K, Sessa WC, Giordano FJ. An engineered VEGF-activating zinc finger protein transcription factor improves blood flow and limb salvage in advanced-age mice. FASEB J. 2006;20(3):479–81.
297. Madou MJ. Fundamentals of microfabrication: the science of miniaturization. 2nd ed. Boca Raton: CRC Press; 2002.
298. Kulig KM, Vacanti JP. Hepatic tissue engineering. Transpl Immunol. 2004;12(3–4):303–10.
299. Burgess KA, Hu HH, Wagner WR, Federspiel WJ. Towards microfabricated biohybrid artificial lung modules for chronic respiratory support. Biomed Microdevices. 2009;11(1):117–27.
300. Tan CP, Seo BR, Brooks DJ, Chandler EM, Craighead HG, Fischbach C. Parylene peel-off arrays to probe the role of cell-cell interactions in tumour angiogenesis. Integr Biol (Camb). 2009;1(10):587–94.
301. Wang YC, Ho CC. Micropatterning of proteins and mammalian cells on biomaterials. FASEB J. 2004;18(3):525–7.
302. Peterson SL, McDonald A, Gourley PL, Sasaki DY. Poly(dimethylsiloxane) thin films as biocompatible coatings for microfluidic devices: cell culture and flow studies with glial cells. J Biomed Mater Res A. 2005;72(1):10–8.
303. Wang GJ, Ho KH, Hsu SH, Wang KP. Microvessel scaffold with circular microchannels by photoresist melting. Biomed Microdevices. 2007;9(5):657–63.
304. Thakar RG, Ho F, Huang NF, Liepmann D, Li S. Regulation of vascular smooth muscle cells by micropatterning. Biochem Biophys Res Commun. 2003;307(4):883–90.
305. Vickerman V, Blundo J, Chung S, Kamm R. Design, fabrication and implementation of a novel multi-parameter control microfluidic platform for three-dimensional cell culture and real-time imaging. Lab Chip. 2008;8(9):1468–77.
306. Gillette BM, Jensen JA, Tang B, Yang GJ, Bazargan-Lari A, Zhong M, Sia SK. *In situ* collagen assembly for integrating microfabricated three-dimensional cell-seeded matrices. Nat Mater. 2008;7(8):636–40.
307. De Silva MN, Desai R, Odde DJ. Micro-patterning of animal cells on PDMS substrates in the presence of serum without use of adhesion inhibitors. Biomed Microdevices. 2004;6(3):219–22.
308. Leclerc E, Baudoin R, Corlu A, Griscom L, Luc Duval J, Legallais C. Selective control of liver and kidney cells migration during organotypic cocultures inside fibronectin-coated rectangular silicone microchannels. Biomaterials. 2007;28(10):1820–9.
309. Nahmias Y, Arneja A, Tower TT, Renn MJ, Odde DJ. Cell patterning on biological gels via cell spraying through a mask. Tissue Eng. 2005;11(5–6):701–8.
310. Kobayashi A, Miyake H, Hattori H, Kuwana R, Hiruma Y, Nakahama K, Ichinose S, Ota M, Nakamura M, Takeda S, Morita I. *In vitro* formation of capillary networks using optical lithographic techniques. Biochem Biophys Res Commun. 2007;358(3):692–7.
311. Makamba H, Kim JH, Lim K, Park N, Hahn JH. Surface modification of poly(dimethylsiloxane) microchannels. Electrophoresis. 2003;24(21):3607–19.
312. Rozkiewicz DI, Kraan Y, Werten MW, de Wolf FA, Subramaniam V, Ravoo BJ, Reinhoudt DN. Covalent microcontact printing of proteins for cell patterning. Chemistry. 2006;12(24):6290–7.
313. Ni M, Tong WH, Choudhury D, Rahim NA, Iliescu C, Yu H. Cell culture on MEMS platforms: a review. Int J Mol Sci. 2009;10(12):5411–41.
314. Bettinger CJ, Langer R, Borenstein JT. Engineering substrate topography at the micro- and nanoscale to control cell function. Angew Chem Int Ed Engl. 2009;48(30):5406–15.
315. Shen JY, Chan-Park MB, He B, Zhu AP, Zhu X, Beuerman RW, Yang EB, Chen W, Chan V. Three-dimensional microchannels in biodegradable polymeric films for control orientation and phenotype of vascular smooth muscle cells. Tissue Eng. 2006;12(8):2229–40.
316. Co CC, Wang YC, Ho CC. Biocompatible micropatterning of two different cell types. J Am Chem Soc. 2005;127(6):1598–9.
317. Tan W, Desai TA. Microscale multilayer cocultures for biomimetic blood vessels. J Biomed Mater Res A. 2005;72(2):146–60.
318. Chang CC, Hoying JB. Directed three-dimensional growth of microvascular cells and isolated microvessel fragments. Cell Transplant. 2006;15(6):533–40.
319. Sudo R, Chung S, Zervantonakis IK, Vickerman V, Toshimitsu Y, Griffith LG, Kamm RD. Transport-mediated angiogenesis in 3D epithelial coculture. FASEB J. 2009;23(7):2155–64.

320. Mironov V, Reis N, Derby B. Review: bioprinting: a beginning. Tissue Eng. 2006;12(4):631–4.
321. Fernandez JG, Mills CA, Martinez E, Lopez-Bosque MJ, Sisquella X, Errachid A, Samitier J. Micro- and nanostructuring of freestanding, biodegradable, thin sheets of chitosan via soft lithography. J Biomed Mater Res A. 2008;85(1):242–7.
322. Wilson WC, Jr., Boland T. Cell and organ printing 1: protein and cell printers. Anat Rec A Discov Mol Cell Evol Biol. 2003;272(2):491–6.
323. Xu T, Jin J, Gregory C, Hickman JJ, Boland T. Inkjet printing of viable mammalian cells. Biomaterials. 2005;26(1):93–9.
324. Moon S, Hasan SK, Song YS, Xu F, Keles HO, Manzur F, Mikkilineni S, Hong JW, Nagatomi J, Haeggstrom E, Khademhosseini A, Demirci U. Layer by layer three-dimensional tissue epitaxy by cell-laden hydrogel droplets. Tissue Eng Part C Methods. 2010;16(1):157–66.
325. Smith CM, Stone AL, Parkhill RL, Stewart RL, Simpkins MW, Kachurin AM, Warren WL, Williams SK. Three-dimensional bioassembly tool for generating viable tissue-engineered constructs. Tissue Eng. 2004;10(9–10):1566–76.
326. Mironov V, Visconti RP, Kasyanov V, Forgacs G, Drake CJ, Markwald RR. Organ printing: tissue spheroids as building blocks. Biomaterials. 2009;30(12):2164–74.
327. Norotte C, Marga FS, Niklason LE, Forgacs G. Scaffold-free vascular tissue engineering using bioprinting. Biomaterials. 2009;30(30):5910–7.
328. Nunes SS, Rekapally H, Chang CC, Hoying JB. Vessel arterial-venous plasticity in adult neovascularization. PLoS One. 2011;6(11):e27332.
329. Hansen-Smith FM. Capillary network patterning during angiogenesis. Clin Exp Pharmacol Physiol. 2000;27(10):830–5.
330. Segal SS. Regulation of blood flow in the microcirculation. Microcirculation. 2005;12(1):33–45.
331. Qutub AA, Mac Gabhann F, Karagiannis ED, Vempati P, Popel AS. Multiscale models of angiogenesis. IEEE Eng Med Biol Mag. 2009;28(2):14–31.
332. Liotta LA, Saidel GM, Kleinerman J. Diffusion model of tumor vascularization and growth. Bull Math Biol. 1977;39(1):117-28.
333. Anderson AR, Chaplain MA. Continuous and discrete mathematical models of tumor-induced angiogenesis. Bull Math Biol. 1998;60(5):857–99.
334. Levine HA, Sleeman BD, Nilsen-Hamilton M. A mathematical model for the roles of pericytes and macrophages in the initiation of angiogenesis. I. The role of protease inhibitors in preventing angiogenesis. Math Biosci. 2000;168(1):77–115.
335. Levine HA, Pamuk S, Sleeman BD, Nilsen-Hamilton M. Mathematical modeling of capillary formation and development in tumor angiogenesis: penetration into the stroma. Bull Math Biol. 2001;63(5):801–63.
336. Arakelyan L, Vainstein V, Agur Z. A computer algorithm describing the process of vessel formation and maturation, and its use for predicting the effects of anti-angiogenic and anti-maturation therapy on vascular tumor growth. Angiogenesis. 2002;5(3):203–14.
337. Mantzaris NV, Webb S, Othmer HG. Mathematical modeling of tumor-induced angiogenesis. J Math Biol. 2004;49(2):111–87.
338. Shiu YT, Weiss JA, Hoying JB, Iwamoto MN, Joung IS, Quam CT. The role of mechanical stresses in angiogenesis. Crit Rev Biomed Eng. 2005;33(5):431–510.
339. Peirce SM. Computational and mathematical modeling of angiogenesis. Microcirculation. 2008;15(8):739–51.
340. Peirce SM, Mac Gabhann F, Bautch VL. Integration of experimental and computational approaches to sprouting angiogenesis. Curr Opin Hematol. 2012;19(3):184–91.
341. Bentley K, Gerhardt H, Bates PA. Agent-based simulation of notch-mediated tip cell selection in angiogenic sprout initialisation. J Theor Biol. 2008;250(1):25–36.
342. Stefanini MO, Qutub AA, Mac Gabhann F, Popel AS. Computational models of VEGF-associated angiogenic processes in cancer. Math Med Biol. 2012;29(1):85–94.
343. Bauer AL, Jackson TL, Jiang Y. A cell-based model exhibiting branching and anastomosis during tumor-induced angiogenesis. Biophys J. 2007;92(9):3105–21.
344. Secomb TW, Alberding JP, Hsu R, Dewhirst MW, Pries AR. Angiogenesis: an adaptive dynamic biological patterning problem. PLoS Comput Biol. 2013;9(3):e1002983.
345. Pries AR, Secomb TW. Modeling structural adaptation of microcirculation. Microcirculation. 2008;15(8):753–64.
346. Pries AR, Secomb TW. Origins of heterogeneity in tissue perfusion and metabolism. Cardiovasc Res. 2009;81(2):328–35.
347. Beard DA, Bassingthwaighte JB. Modeling advection and diffusion of oxygen in complex vascular networks. Ann Biomed Eng. 2001 Apr;29(4):298-310.
348. Godde R, Kurz H. Structural and biophysical simulation of angiogenesis and vascular remodeling. Dev Dyn. 2001 Apr;220(4):387-401.
349. Griffith LG, Swartz MA. Capturing complex 3D tissue physiology *in vitro*. Nat Rev Mol Cell Biol. 2006;7(3):211–24.
350. Pedersen JA, Lichter S, Swartz MA. Cells in 3D matrices under interstitial flow: effects of extracellular matrix alignment on cell shear stress and drag forces. J Biomech. 2010;43(5):900–5.
351. Jain RK, Tong RT, Munn LL. Effect of vascular normalization by antiangiogenic therapy on interstitial hypertension, peritumor edema, and lymphatic metastasis: insights from a mathematical model. Cancer Res. 2007 Mar 15;67(6):2729-35.
352. Fung YC. A first course in continuum mechanics. 3rd Ed. Upper Saddle River (NJ): Prentice-Hall, Inc; 1994.
353. Dickinson RB, Guido S, Tranquillo RT. Biased cell migration of fibroblasts exhibiting contact guidance in oriented collagen gels. Ann Biomed Eng. 1994;22(4):342–56.

354. Maroudas NG. Chemical and mechanical requirements for fibroblast adhesion. Nature. 1973;244(5415):353–4.
355. Namy P, Ohayon J, Tracqui P. Critical conditions for pattern formation and *in vitro* tubulogenesis driven by cellular traction fields. J Theor Biol. 2004;227(1):103–20.
356. Guilkey JE, Hoying JB, Weiss JA. Computational modeling of multicellular constructs with the material point method. J Biomech. 2006;39(11):2074–86.
357. Qutub AA, Popel AS. Elongation, proliferation & migration differentiate endothelial cell phenotypes and determine capillary sprouting. BMC Syst Biol. 2009;3:13