Chapter 6
Structure, Function, and Development of Blood Vessels: Lessons for Tissue Engineering

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The establishment of blood vessel networks is a matter of life and death for tissues and organisms. Failure to form a functional vascular network causes early death of embryos, and also dysfunction of ECs contributes to many diseases, including stroke, thrombosis, and atherosclerosis. Furthermore, there is a considerable clinical need for alternatives to the autologous vein and artery tissues used for vascular reconstructive surgeries such as lower limb bypass, arteriovenous shunts, and repairs of congenital defects to the pulmonary outflow tract. So far, synthetic materials, particularly in small-diameter applications, have not matched the efficacy of native tissues. Therefore, substantial resources are being directed toward research into the cellular, molecular, and physical factors that regulate the formation, stability, and functional responses of the vasculature. While academic research in the field of tissue engineering in general has been active, yet there has been no clear example of clinical and commercial success. The recent transition of cell-based therapies from experimental to clinical use, however, is a breakthrough in the field of cardiovascular tissue engineering.

Here, we discuss the structure of blood vessels and key signaling molecules, which play significant role in vasculogenesis, angiogenesis, and maturation of nascent blood vessels in Sects. 6.1 and 6.2, respectively. The discussion is followed by a description of promising approaches specific to tissue-engineered blood vessels and a brief introduction to some clinical results in Sect. 6.4. But before we explain about tissue engineering approaches, finding the appropriate

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sources of ECs is of utmost importance, which is discussed in Sect. 6.3. The unique regulatory, reimbursement, and production challenges facing personalized medicine are also discussed in the last Sect. 6.5.

6.1 Structure of Blood Vessels

Delivery of nutrients and other molecules as well as blood and immune cells to all tissues in our body is done by blood vessels. Nascent vessels consist of a tube of ECs that mature into three specialized structures: *capillaries*, *arteries,* and *veins*. ECs and mural cells that are surrounded by extracellular matrix (ECM) comprise the walls of vessels.

To form mature blood vessels, immature blood vessels formed by vasculogenesis and angiogenesis must mature at two levels: the *level of vessel wall* as well as *network level*. *Vessel wall* maturation is the result of mural cell recruitment, development of surrounding matrix and elastic laminae, and organ specific specialization of ECs, mural cells, and matrix. Optimal patterning of network by branching, expanding, and pruning to meet local demands leads to *network-level* maturation [1, 2].

6.1.1 Capillaries

The most abundant vessels in our body are capillaries. Capillaries consist of ECs covered with a sparse layer of pericytes that is surrounded by basement membrane (BM). Because of their wall structure and large surface-area-to-volume ratio, these vessels form the main site of exchange of nutrients between blood and tissue. Depending upon the organ or tissue, the capillary endothelial layer could be continuous (as in muscle), fenestrated (as in kidney or endocrine glands), or discontinuous (as in liver sinusoids). The endothelia of the blood–brain barrier or blood–retina barrier are further specialized to include tight junctions and are thus impermeable to various molecules [1].

6.1.2 Arterioles and Venules

Arterioles and venules are small-diameter blood vessels in the microcirculation that extend and branch out from an artery and vein, respectively, which lead to capillaries [1]. These vessels have an increased coverage of mural cells compared with capillaries. Pre-capillary arterioles are completely invested with vascular SMCs that form their own BM and are circumferentially arranged, closely packed and tightly associated with the endothelium. Extravasation of macromolecules and cells from the blood stream typically occurs from postcapillary venules [2, 3].

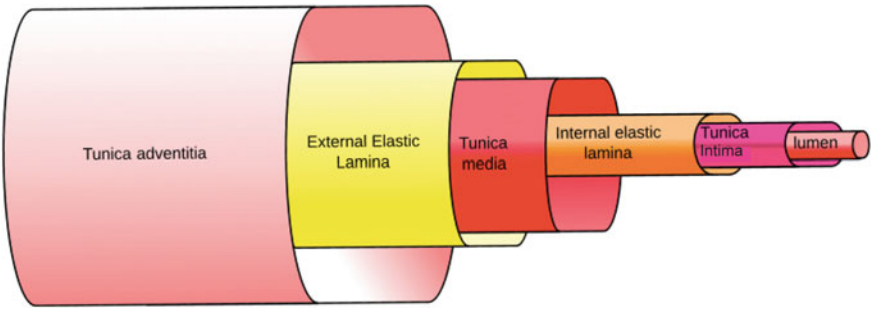

Fig. 6.1 Schematic representation of various layers in blood vessels

6.1.3 Arteries and Veins

Arteries are blood vessels that carry blood away from the heart. This blood is normally oxygenated, with the exception of the pulmonary and umbilical arteries. Veins are blood vessels that carry blood toward the heart. Most veins carry deoxygenated blood from the tissues back to the heart; exceptions are the pulmonary and umbilical veins, both of which carry oxygenated blood to the heart. Larger vessels, arteries and veins, consist of three specialized layers as shown in Fig. 6.1: tunica intima, the tunica media, and the tunica adventitia. The tunica intima is made of a monolayer of ECs with an underlying BM. The tunica media is generally composed of a dense population of concentrically organized SMCs and is separated from the tunica intima by an internal elastic lamina. The tunica adventitia forms the external layer and contains fibroblast cells, collagenous ECM, nerves, and vasa vasorum [4]. The vascular wall ECM composed of structural proteins like collagen and elastin and adhesion proteins like fibronectin and laminin, as well as glycosaminoglycans (GAGs), proteoglycans, growth factors, cytokines, and matrix-degrading enzymes and their inhibitors [5, 6].

6.2 Important Signaling Molecules in Vasculogenesis and Angiogenesis

Understanding important signaling molecules at each level of development of arteries, veins and capillaries and their maturation could help us to engineer blood vessels that are a more faithful mimic of the natural system. Here, we review briefly the important molecules and pathways in vasculogenesis, angiogenesis, and maturation. Tissue engineering strategies may benefit from generating materials that can guide these biological events in the formation of vascular networks. How scientists and researchers use this information to engineer the blood vessels is discussed in Sect. 6.4.

6.2.1 Formation of Immature Vasculature by Vasculogenesis and Angiogenesis

During embryonic development, angioblasts migrate to various regions of the developing embryo and differentiate into ECs in response to local cues such as growth factors and ECM components. The ECs then form a vascular plexus, which is a network built by connections (anastomoses) between blood vessels. This process is called vasculogenesis and it is not limited to the embryonic period, as a similar process can also occur in adults through the recruitment and participation of bone marrow-derived endothelial progenitor cells [3, 7, 8].

Angiogenesis is another mechanism of blood vessel formation that occurs through the sprouting of existing blood vessels. Angiogenesis is a sequential, multistep process that begins with activation of a quiescent endothelium by pro-angiogenic factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and angiopoietin-2 (Ang2) that are often produced by hypoxic or tumorigenic tissues [3, 8]. Hypoxia up-regulates expression of a number of genes involved in vessel formation, patterning, and maturation, including nitric oxide synthase, VEGF, and Ang2. Nitric oxide, which is a product of nitric oxide synthase, dilates vessels and make them more responsive to VEGF because they becomes more leaky. Ang2 also facilitates sprout formation in the presence of VEGF. The sprouts anastomose to form vascular loops and networks [3].

Angiogenesis is also dependent on degradation of the BM, a thin layer of ECM between the epithelial cell layer and the endothelial cell lining of blood vessels. Degradation of BM is due to up-regulation of matrix metalloproteinases (MMPs) such as MMP2, MMP3, and MMP9, and suppression of protease inhibitors such as tissue inhibitor of metalloproteinase-2 (TIMP2). BM degradation is followed by migration of an endothelial tip cell from the leading edge of a vascular sprout; this leading edge defines the direction of the newly growing sprout [2, 6, 7].

6.2.2 Endothelial Cell Branching and Proliferation

High levels of proangiogenic factors (such as VEGFA and VEGFC) and of VEGF receptor 2 (VEGFR2) or VEGFR3 signaling select “tip cells” (TCs) for sprouting during angiogenesis. By contrast, Delta-like 4-notch signaling laterally inhibits TC fate in adjacent ECs. TC sprouting behavior is facilitated by the vascular endothelial cadherin-mediated loosening of EC–EC junctions, matrix metalloproteinase-mediated degradation of ECM and the detachment of pericytes. Guidance of TC sprouting is due to the gradients of proangiogenic growth factors and various environmental guidance cues, such as semaphorins and ephrins. During sprout elongation, TCs are trailed by endothelial “stalk cells” (SCs), which maintain connectivity with parental vessels and initiate partitioning-defective3 (PAR3)-mediated vascular lumen morphogenesis. Expression of VEGFR1 and activation of

notch, Roundabout homologue 4 and WNT signaling in SCs repress TC behavior to maintain the hierarchical organization of sprouting ECs. However, TCs and SCs may also shuffle and exchange positions during angiogenic sprouting. Upon contact with other vessels, TC behavior is repressed and vessels fuse by the process of anastomosis, which is assisted by associated myeloid cells. The ECs adjacent to the tip cells begin to proliferate and elongate to form capillary sprouts, which then assemble to form a vessel lumen. After the activation and proliferation stages, a nascent blood vessel must mature to become functional [6–11].

6.2.3 Stabilization of Immature Vasculature

After the activation and proliferation stages, a nascent blood vessel must mature to become functional. The nascent vessels are stabilized by recruiting mural cells and by deposition of an ECM, in a process known as arteriogenesis. There are at least four molecular pathways that regulate this process, including the following:

1. Platelet-derived growth factor PDGFB and PDGF receptor (PDGFR)-β.
2. Sphingosine-1-phosphate-1 (S1P)-endothelial differentiation sphingolipid G-protein-coupled receptor-1 (EDG1).
3. Ang1-Tie2.
4. Transforming growth factor TGF-β1.

Recruitment of mural cells such as pericytes and smooth muscle cells (SMCs) to the developing immature vasculature by platelet-derived growth factor B (PDGFB) and transforming growth factor-β1 (TGF-β1) stabilize the vessel wall [2, 7, 12, 13]. The contact between ECs and mural cells is strengthened by bioactive lipid spingosine1 phosphate (S1P) through activating the guanine nucleotide-binding-coupled receptor, and S1P receptor 1(S1PR1 or EDG1) signaling [7, 14]. Tie receptors (Tie1 and Tie2) and their ligands Ang1 and Ang2 are also critical for vessel formation and stabilization. Main sources of Ang1 and Ang2 are the mural cells and ECs, respectively. Ang1 stabilizes nascent vessels by facilitating communication between ECs and mural cells. Ang2 acts as an antagonist of Ang1 in the absence of VEGF and destabilizes vessels in the presence of VEGF [15].

TGF-β1 is a multifunctional growth factor that promotes vessel maturation by stimulating ECM production and by inducing differentiation of mesenchymal cells to mural cells. It is expressed in a number of cell types, including ECs and mural cells, and depending on the context and concentration, could be pro- or anti-angiogenic. Recent in vitro studies indicate that the TGF-β1–ALK1 pathway is a positive regulator of endothelial cell migration and proliferation by inducing ECs and fibroblasts to express Id1, a protein required for proliferation and migration. On the other hand, the TGF-β1–ALK5 pathway is a positive regulator of vessel maturation by inducing the plasminogen activator inhibitor (PAI1) in ECs. PAI1

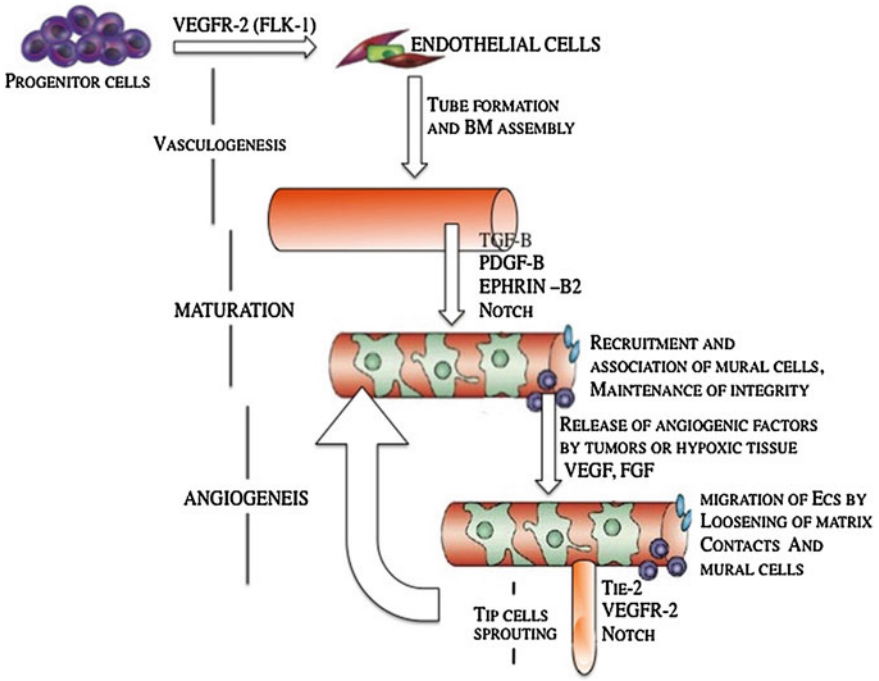

Fig. 6.2 Schematic of important signaling molecules that are involved in vasculogenesis, maturation of blood vessels, and angiogenesis, modified from Ref. [6]

promotes vessel maturation by preventing degradation of the provisional matrix around the nascent vessel. Thus, the degree to which TGF-β signals through ALK1 versus ALK5 can determine the pro- or anti-angiogenic effect of TGF-β [2, 10]. Figure 6.2 shows important signaling molecules that are involved in vasculogenesis, maturation of blood vessels and angiogenesis.

6.2.3.1 Role of Basement Membrane in Stabilization of Immature Blood Vessels

As mentioned earlier, BM degradation happens during the complicated multistep angiogenesis process. Direct contact between the BM and the EC layer provides important signals that control the stability of EC layer and facilitate EC tube stabilization. Vascular basement membrane matrices are largely composed of structural components, including laminin (particularly laminin IV), collagen IV, and fibronectin. Other proteins provide bridging functions such as nidogens 1 and 2, and the heparan sulfate proteoglycan perlecan, which facilitate the co-assembly of BM components [16–18]. It has long been known that ECs have the capacity to synthesize most if not all of these proteins, so it was generally assumed that BM

assembly occurred through ECs alone. However, in a variety of tissues, most notably the skin, it is clear that BM assembly requires more than keratinocytes and was strongly stimulated by the presence of fibroblasts in collagenous matrices underlying the keratinocyte layer. Thus, by analogy with these findings, it is likely that vascular basement membrane assembly may require heterotypic cell–cell contacts, which was originally suggested by Davis and Senger [19]. A recent study by Stratman et al. demonstrates that pericyte recruitment to EC-lined tubes in vitro and in vivo is necessary to stimulate vascular basement membrane matrix assembly, a key step in vascular maturation and stabilization [20].

Collectively, the matrix serves as a store for various growth factors and proenzymes involved in vessel development. The balance between proteases (such as MMP2, MMP3, MMP9, and urokinase plasminogen activator) and their inhibitors (such as tissue inhibitors of metalloproteinases and PAI1) controls BM and ECM degradation and could influence EC and mural cell migration [7, 20, 21]. These proteases also lead to the release of various proangiogenic growth factors, such as VEGF and basic fibroblast growth factor (bFGF), which are sequestered in the matrix. Protease activity can also generate anti-angiogenic molecules by cleaving plasma proteins (such as angiostatin from plasminogen), matrix molecules (such as tumstatin from collagen type IV), or the proteases themselves (such as PEX from MMP2). Thus, branching patterns of vessels are tightly regulated by spatial and temporal concentration profiles of growth factors and protein fragments that transport and bind to the matrix [2, 4].

6.3 Sources of Endothelial Cells and Their Progenitors

One potential source of ECs are embryonic stem cells, which are pluripotent and thus capable of differentiating into all cell types of the endoderm, ectoderm, and mesoderm. Although ESCs have the advantages of greater proliferative capacity and pluripotentiality when compared to other endothelial cell precursors, these properties also raise a concern. Specifically, the inadvertent administration of an undifferentiated (and thus pluripotent) ESC to a patient would risk teratoma formation. Accordingly, the clinical development of this cell therapy will require robust differentiation and purification protocols, supported by data showing the safety of these cells. The therapeutic use of these cells is further complicated because they are allogeneic and therapeutic engraftment may require immunosuppression, which carries additional risk. Finally, the clinical use of these cells may be influenced by the ethical debate surrounding the isolation of cells from human embryos. Accordingly, there is great interest in a new form of pluripotential cell that can obviate some of these concerns. Induced pluripotent stem cells (iPSCs) can be “reprogrammed” from adult somatic cells using a variety of methods, established primarily by Yamanaka and Thomson. iPSCs have the potential to generate patient-specific tissues for disease modeling and regenerative medicine applications. However, before iPSC technology can progress to the

translational phase, several obstacles must be overcome. These include uncertainty regarding the ideal somatic cell type for reprogramming, the low kinetics and efficiency of reprogramming, and karyotype discrepancies between iPSCs and their somatic precursors. In this section, we describe different sources of ECs and common endothelial cell markers that have been investigated so far by researchers, from embryonic and iPSCs to adult cells.

6.3.1 Embryonic Stem Cell-Derived Endothelial Cells

- *From Embryoid Bodies (EB)*
  Mouse embryonic stem cells (mESCs) can differentiate into hemangioblasts after forming embryoid bodies (EBs). Hemangioblasts that form blood islands contain endothelial and haematopoietic progenitors [22]. Formation of a vessel-like network is the result of further differentiation of the EBs [23]. Moreover, it has been shown that endothelial cell markers differentiated from EBs are expressed in the same order that is expressed in endothelial differentiation during embryonic development [24].
  During human EB differentiation, endothelial cell markers such as CD31, CD34, VE-cad ,and GATA-2 show increasing trends in their expression [25, 26]. CD31, CD34, and VE-cad reach a maximum at days 13–15 and GATA-2 around day 18. Other endothelial markers such as VCAM1, FLT-1, FLT-2; vasculogenic growth factors such as VEGF, Ang1, Ang2, and PDGF; and transcription factors such as GATA1 and GATA3 are up-regulated as well [27]. Like mouse ESCs, human ESCs (hESCs) can spontaneously differentiate and organize within EBs into three-dimensional vessel-like structures, in a pattern that resembles embryonic vascularization. The capillary area in the human EBs increases during subsequent maturation steps, starting from cell clusters that later sprout into capillary-like structures and eventually organize in a network-like arrangement. These isolated $CD31^+$ cells from human EBs (days 13–15), express endothelial markers and can form vascular tubes in vitro and in vivo [28].
- *On feeder layer and ECM*
  Seeding ES cells on feeder cells or within an ECM can also induce differentiation into haematopoietic and endothelial lineages. For example, mouse endothelial progenitors ($Flk\text{-}1^+$ cells) were isolated following the differentiation of ESCs on collagen. The isolated $Flk\text{-}1^+$ cells in mouse systems are precursors for haematopoietic, endothelial, and SMCs and can also give rise to contracting cardiac cells, thus acting as cardiohemangioblasts [29, 30]. Culturing of human embryonic stem cells (hESC) on collagen IV resulted in two types of cell population that differ by size. The smaller cell population showed an upregulation of specific endothelial markers, such as CD31, CD34, Tei2, and GATA2. Cord-like organization of the cells (20 % ECs) was observed by re-plating the smaller population of cells on collagen IV with VEGF supplementation.

Moreover, addition of PDGFB induced differentiation into SMCs [31]. Seeding hESCs on stromal feeder cells (bone marrow and yolk sac) could lead to differentiation into $CD34^+$ cells (1–2 %). Interestingly, about 50 % of the $CD34^+$ cells also express CD31. The $CD34^+$ cells were isolated and differentiated into haematopoietic cells [32].

6.3.2 Adult-Derived Endothelial Cells

Human umbilical vein endothelial cells (HUVECs) show relatively higher proliferative potential among the isolated $CD31^+$ ECs that originate from veins and arteries of different tissues [28]. Isolation of $CD34^+$ and Flk-1^+ cells from peripheral blood using magnetic beads is another source of adult ECs [16]. These isolated progenitor cells could differentiate into ECs and incorporate into neovascularization sites in mouse and rabbit hindlimb ischemic models [34]. $CD34^+$ cells, mobilized from the bone marrow following treatment with granulocyte macrophage colony stimulating factor, improved ventricular function and neoangiogenesis in ischemic nude rat myocardium [35]. $CD133^+$ cells purified from bone marrow were also shown to enhance human myocardial perfusion and global function [36]. Another important source of adult ECs is the umbilical cord blood, which contains more $CD133^+$ and $CD34^+$ cells than adult peripheral blood and has higher proliferation capacity [37].

6.3.3 Induced Pluripotent Stem Cell-Derived Endothelial Cells

Yamanaka et al. have shown systematic differentiation of cardiovascular cells from mouse iPSCs. Induced pluripotent stem (iPS) cells were generated from mouse skin fibroblasts by introducing four transcription factors (Oct3/4, Sox2, Klf4, c-myc), and then, the same approach was applied on ES cells to induce cardiovascular differentiation. They showed that $Flk1^+$ cells could differentiate into artery, vein, and mural cells [38]. Rufaihah et al. have differentiated human iPSCs (hiPSCs) into endothelial cells (hiPSC-ECs) to assess their ability to improve perfusion in a murine model of peripheral arterial disease [39, 40]. In brief, endothelial differentiation was initiated by culturing hiPSCs for 14 days in differentiation media supplemented with bone morphogenetic protein 4 and VEGF. They purified the heterogenous mixture of cells by FACS using an antibody directed against CD31. The purified hiPSC-ECs generated capillary-like structures when grown in Matrigel and incorporated acetylated-LDL cholesterol. These cells expressed endothelial markers such as FLK-1 (KDR), CD31, CD144, and eNOS. When exposed to hypoxia, the hiPSC–ECs produced angiogenic cytokines and growth factors. Subsequently, they transduced the cells with a double-fusion

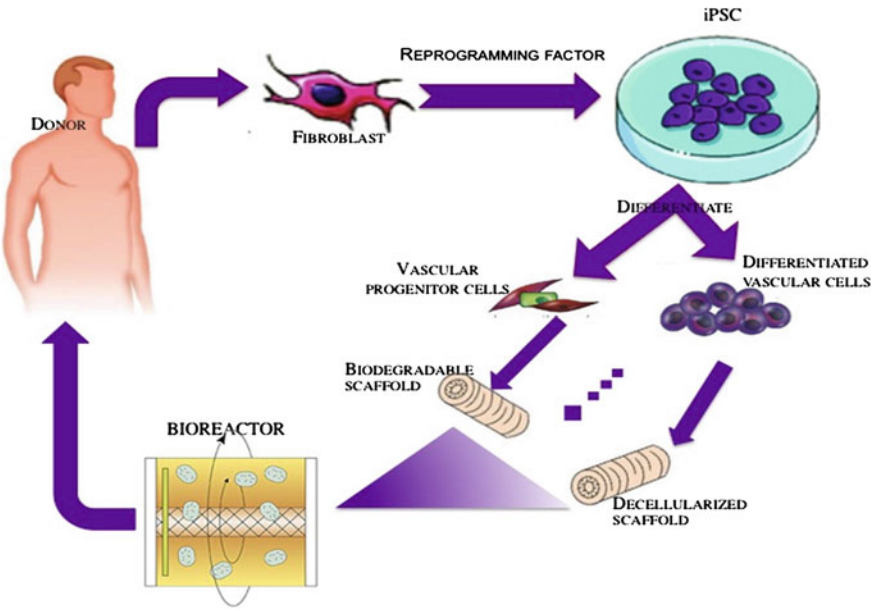

Fig. 6.3 Development of therapeutic cells from human-induced pluripotent cells

construct comprising firefly luciferase for BLI and green fluorescence protein for histochemistry. The hiPSC–ECs were administered on days 0 and 7 after femoral artery ligation into the ischemic hindlimb of immunodeficient mice. Over a two-week period, BLI revealed reduction of cells, but some hiPSC–ECs survived in the ischemic limb for at least 2 weeks. At that time, and for up to 4 weeks, perfusion was improved by over 30 % by comparison to the vehicle-treated group, as assessed by laser Doppler imaging. This effect was associated with a 60 % increase in the total number of capillaries in the ischemic limb of mice receiving hiPSC–EC injections by comparison to the vehicle-treated group. This preclinical work provided proof-of-concept for the use of hiPSC–EC in peripheral arterial disease [41].

Figure 6.3 shows development of therapeutic cells from human-induced pluripotent cells. Readily accessible somatic cells (e.g., skin fibroblasts) are harvested from a patient and expanded in culture. Cells are exposed to reprogramming transcriptional factors, in the form of cell permeant peptides and modified mRNA. The resulting iPSC colonies are differentiated into vascular progenitor cells, that are administered directly to patients with vascular disease, or which are incorporated into matrices as a biological conduit such as cylindrical bioengineered matrix or decellularized cadaveric vessels for surgical implantation. These bioengineered conduits would serve to replace autologous saphenous vein in patients that have insufficient or diseased veins [41].

6.4 Engineering Blood Vessels from Stem Cell-Derived Endothelial Cells: Recent Advances and Applications

In the last three sections, we introduced the structure of blood vessels, important cell signaling molecules in vasculogenesis/angiogenesis, and different cell sources for ECs. Now, we will describe the tissue engineering approaches are available for making macrovessels and microvessels.

6.4.1 Approaches to Macrovessel Tissue Engineering (e.g., Heart Valve)

The approaches to engineer macrovessels can be divided into several distinct categories, although there may be some overlapping features. Successful application of these approaches, either individually or in combination, is expected to enhance therapeutic opportunities by building functional tissue and organ systems for regenerative medicine.

1. Formation of a tissue in vitro by seeding cells on a biodegradable scaffold and maturing a tissue (to be implanted in vivo) in a bioreactor.
2. Cell-seeded natural biodegradable scaffolds.
3. Guided tissue regeneration via implanted degradable tissue that is remodeled by endogenous cells.
4. Implantation of decellularized valvular material.
5. Other approaches such as microfabrication techniques and scaffold-free approaches.

In all of the mentioned approaches, choosing the right cell among the spectrum of stem cells to differentiated cells and also the right scaffold is of utmost importance [42]. The scaffold should provide the initial requisite mechanical strength to withstand in vivo hemodynamic forces until vascular SMCs and fibroblasts reinforce the ECM of the vessel wall. Hence, the choice of scaffold is crucial for providing guidance cues to the cells to behave in the required manner to produce tissues and organs of the desired shape and size. Several types of scaffolds have been used for the reconstruction of blood vessels. They can be broadly classified as biological scaffolds, decellularized matrices, and polymeric biodegradable scaffolds. A review written by Pankajakhshan et al. focuses on the different types of scaffolds that have been designed, developed, and tested for tissue engineering of blood vessels [43].

6.4.1.1 Bioreactor Approach

The bioreactor approach should provide a desirable physiological, metabolic, and mechanical environment for blood vessel formation in vitro. To develop well-developed and effective construct for implantation and remodeling one approach would be to optimize the cellular component, the scaffolds, and the in vitro process conditions. Novel scaffolds are also under investigation. Multiple studies have emphasized that dynamic conditioning using bioreactors that provide a flow regime that mimics that of the intended application enhances construct tissue properties [44, 45]. Oxygen tension might be a key parameter for the achievement of sufficient tissue quality and mechanical integrity in tissue-engineered heart valves [46, 47]. A mesenchymal stem cell-seeded, valve-shaped construct has been assembled from layered collagenous scaffolds. Autologous fibrin-based engineered heart valves showed favorable results both in vitro and in vivo [48, 49]. Culturing vascular cells on polymer scaffolds and subjecting the scaffold to pulsating flow is another approach that is studied by Niklason et al. [50]. In fact, they seeded bovine aortic SMCs into hollow tubular polyglycolic acid (PGA) scaffolds and then injected bovine aortic ECs into the lumen. Compared with native arteries, the engineered arteries demonstrate similarities in wall thickness and collagen content after eight weeks of culture in a bioreactor. A key issue moving forward will be the real-time noninvasive and nondestructive assessment of mechanical properties of engineered heart valves both in vitro and utilization of such techniques in vivo to ensure quality.

6.4.1.2 Decellularized Vascular Material

Removal of all viable cells while preserving the ECM integrity is the goal in producing decellularized xenogeneic tissue. This method provides valuable material for heart valve tissue engineering. Decellularized materials have the advantage of preserving ECM components that may support cell adhesion and molecular sequestering, as well as desirable mechanical properties. However, ECM disruption by the decellularization process and immunogenicity are concerns inherent in the use of decellularized ECMs. For instance, decellularization resulted in substantial microscopic disruption of the ECM, which may negatively impact the durability of heart valve leaflets [51]. Moreover, evaluation of the relative immune responses of different valve components of decellularized porcine aortic valve compared with native and glutaraldehyde fixed valves showed that collagen I elicited a strong response but elastin induced a minimal response [52]. Recellularization of these valves has been reported in an aortic valve replacement model in juvenile pigs [53]. Another study investigated the function, histological changes, potential of in vivo re-endothelialization of decellularized aortic valve allografts in orthotopic position in sheep. The valves exhibited trivial regurgitation and normal morphology with no signs of graft dilatation, degeneration, or rejection [54]. Toxicity that is introduced to scaffold by the chemicals used in the decellularization process is another concern in decellularized natural ECMs. A clinical study investigated the safety and

effectiveness of the Ross procedure (pulmonary to aortic valve autograft) using a decellularized, fibronectin-coated pulmonary valve allograft or xenograft seeded with autologous vascular ECs from a forearm or saphenous vein to reconstruct the right ventricular outflow tract. These valves showed excellent hemodynamic performance during midterm follow-up [55]. In contrast, recurrent right-sided heart failure after the Ross procedure was reported in a study done by Hiemann et al. [56].

6.4.1.3 Cell-Seeded Natural Biodegradable Scaffold

The cell-seeded natural biodegradable scaffold approach involves isolating and growing ECs on polymer scaffolds in vitro, followed by in vivo implantation. This method has been tested in an ovine model, where expanded pulmonary arterial cells were grown on polyglactin/poly (lactic-co-glycolic acid) tubular scaffolds for one week before transplantation into pulmonary arteries of lamb. Over a period of 24 weeks, the vascular grafts showed growth and development of endothelial lining and the production of ECM components, such as collagen and elastin fibers [57].

6.4.1.4 Microfabrication Technique

Another approach for the in vitro induction of endothelial networks in engineered tissue constructs is to prefabricate scaffolds to include channels that later could be lined with ECs. Microfabrication techniques are currently under way to engineer such network structures that will mimic the capillary network, expanding from a main vessel (like arteries), and merging back to a single vessel (like veins). In such systems, endothelial cells are seeded into the channel network and their attachment and behavior under flow are analyzed. Inkjet printing can be used to pattern cells into tubular structures. This technique, as well as other cell-printing techniques such as laser-guided direct writing, could be used in the future for the assembly of complex vascularized tissues. In one such approach, Tien and coworkers developed a method to prefabricate hollow channels within collagen gels. ECs were then seeded along the interior of the channel such that they formed a vessel-like structure that permitted flow of solution through the tube lumen. The authors demonstrated endothelial barrier function and appropriate barrier breakdown upon exposure to inflammatory cytokines [58].

6.4.1.5 Cell-Synthesized ECM-Scaffold-Free Approach

Although biomaterials-based solutions are promising, there are challenges that need to be solved. Some of the key issues that may affect the long-term behavior of the engineered tissue construct, and directly interfere with its primary biological function, are scaffold choice, immunogenicity, degradation rate, toxicity of degradation products, host inflammatory responses, fibrous tissue formation due to

scaffold degradation, and mechanical mismatch with the surrounding tissue. To address these problems, fabrication techniques for production of scaffold-free engineered tissue constructs have recently emerged. Norotte et al. reported a fully biological self-assembly approach, which was implemented through a rapid prototyping bioprinting method for scaffold-free small-diameter vascular reconstruction. Various vascular cell types, including SMCs and fibroblasts, were aggregated into discrete units, either multicellular spheroids or cylinders of controllable diameter (300–500 mm). These were printed layer-by-layer concomitantly with agarose rods, used as a molding template. A unique aspect of the method is the ability to engineer vessels of distinct shapes and hierarchical trees that combine tubes of distinct diameters. The technique is rapid and scalable [59].

In another study done by L'Heureux et al. the SMCs and fibroblasts were cultured in medium containing sodium ascorbate for increased ECM deposition. After 1 month of in vitro culture, the sheet of SMCs in their own ECM was wrapped around a tube, covered with a sheet of fibroblasts in their own ECM, and then, the luminal surface was seeded with ECs. This construct reportedly had burst strength of over 2,000 mm Hg. In addition, the SMCs expressed desmin, and the ECs strongly inhibited platelet adhesion in vitro. However, when these grafts were implanted in dogs as a canine femoral arterial interposition graft, they had a patency rate of approximately 50 %. In addition, the grafts required 3 months for production. This approach has been further tested in three patients undergoing hemodialysis. The vessels constructed from autologous dermal fibroblasts and ECs were implanted as arteriovenous fistulas for dialysis access and were allowed to mature in vivo before use. During five months of implantation, no failures were observed, and the grafts functioned well. Although the results are encouraging, this approach requires long culture and maturation periods that would limit the application of these vessels in urgent cases [60, 61].

6.4.2 *Approaches to Microvessel Tissue Engineering (e.g., Capillaries)*

Incorporation of a microcirculation into engineered tissues presents multiple challenges, including the formation of microscale vascular conduits for blood flow, a functional endothelium that regulates vascular activity, and specialized cell types that perform the physiological function of the tissue of interest. Several approaches have been developed to address these challenges, including the following:

1. Incorporation of biomolecular cues within the material.
2. Seeding of vascular or vascular-inducing cells in the scaffold.
3. Use of microfabrication technologies to engineer branched microfluidic channels within biocompatible materials.

Here, we discuss each of the above-mentioned approaches in more detail.

6.4.2.1 Incorporation of Biomolecular Cues Within the Material

Growth factor incorporation in three-dimensional (3D) engineered tissues is crucial for angiogenesis and vascularization. In order to establish a functional microvascular network, coordinated delivery of several key factors such as VEGF, FGFs, PDGFs, TGFs, angiopoietins, ephrins, placental growth factors and various chemokines are beneficial. Presentation of a single factor such as VEGF is not typically sufficient to form functional conduits and can, in contrast, lead to induction of tortuous and leaky vessels. Thus, spatial and temporal regulation of growth factor signaling may help ensure accurate vessel growth and remodeling [62, 63].

The need for materials that could be chemically and mechanically tailored to incorporate and release bioactive molecule, while meeting biocompatibility and biodegradability standards, has led to further use of hydrogels as scaffolds for engineered tissue constructs. Hydrogels are hydrated materials made from a cross-linked network of hydrophilic polymers. The integration of factors that induce rapid endothelial cell ingrowth and that stabilize the vascular network as it forms could support the success of these hydrogels [6]. We discuss growth factor-releasing hydrogels and protease-sensitive hydrogels in Tissue Engineering Approaches to Deliver Growth Factors and Protease-Sensitive Hydrogels, respectively. ECM peptides that induce vasculogenesis/angiogenesis in hydrogels are discussed in ECM Peptides Used in the Hydrogel Backbone to Induce Angiogenesis and Vasculogenesis. These discussions are followed by the effect of hydrogel stiffness and porosity on angiogenesis/vasculogenesis (Hydrogel Stiffness and Porosity).

Tissue Engineering Approaches to Deliver Growth Factors

Strategies for biomaterial presentation of growth factors in tissue engineering can be conceptually divided into two areas:

1. Chemical immobilization (covalent or noncovalent) of the growth factor into or onto the matrix; and
2. Physical encapsulation of growth factors in the delivery system.

The first approach typically involves chemical binding or affinity interaction between the growth factor-containing polymer and a cell or a tissue. The second approach is achieved by the encapsulation, diffusion, and pre-programmed release of growth factor from substrate into the surrounding tissue. There are several methods for each approach that are described in detail in a review by Mooney and coworkers [64].

Investigators have developed a wide range of ECM-mimicking biomaterials to immobilize growth factors or growth factor mimics, including hydrogels containing ligands from fibronectin, laminin, collagen, elastin or the GAGs heparin

sulfate, chondroitin sulfate, hyaluronic acid, or a variety of synthetic hydrogels [64]. In a study by Hubble and coworkers, a rationally designed combinatorial approach was used to discover a sulfated tetra-peptide that binds to VEGF. SY(SO3)DY(SO3) was identified as the top binder to VEGF, which mimics heparin binding to VEGF as a potential improvement over natural heparin [65]. Koch et al. similarly used a synthetic homo-bifunctional polymer cross-linker, disuccinimidyl disuccinate poly (ethylene glycol), to attach VEGF to collagen matrices [66]. PEG-ephrinA1 immoblized to hydrogel surfaces induced endothelial cell tubulogenesis with luminal diameters in the range of 5–30 μm, creating structures resembling early-stage capillaries [67]. In another study, PEG-VEGF not only increased endothelial cell tubulogenesis, but also increased endothelial cell motility 14-fold and cell–cell connections 3-fold in a three-dimensional, biodegradable hydrogel [68].

Saik et al. developed MMP-sensitive PEGDA hydrogels immobilized with ephrin A1 ligands, which stimulate a wide range of receptors that induce vascularization. The efficacy of ephrin A1 ligand conjugation was demonstrated 14 days after implantation, by comparing vascular network parameters such as vessel density, branch points of MMP-sensitive, PDGF-BB-containing PEGDA hydrogels with or without immobilized ligand. The biodegradable, bioactive hydrogels immobilized with ephrin A1 ligand produced a denser vasculature in the mouse cornea pocket relative to the nonligand-containing scaffold [69].

Transfecting cells to overexpress angiogenic growth factor genes is another way to promote prolonged, local growth factor delivery [70, 71]. In one example, mononuclear cells, which give rise to endothelial progenitor cells, were transfected to overexpress VEGF. Transfection with a VEGF-encoding gene stimulated their differentiation into ECs for vasculogenesis, while also enhancing local angiogenesis [70]. Myoblasts have also been transfected to express VEGF and bFGF to improve vascularity in engineered muscle tissue [72].

Protease-Sensitive Hydrogels

Cell-induced proteolysis is often required for 3D cell migration and invasion, because the porosity of the ECM may lead to barrier function and thus inhibit migration [73]. Whereas many synthetic biomaterials have been designed to degrade by ester hydrolysis, such nonenzymatic hydrolysis of matrices is not cell mediated and is less directly responsive to cell-mediated remodeling and tissue morphogenesis. Cell-induced proteolysis is a reciprocal interaction between ECM and the cells, as the ECM stimulates the cells and the cellular proteases remodel the ECM and release associated bioactive components from it. For example, Phelps et al. seeded NIH3T3 fibroblasts in poly (ethylene glycol)-diacrylate (PEGDA)-based degradable scaffold and incorporated some responsive elements, such as protease-labile cross-links, cell adhesive peptides, and conjugated VEGF. They demonstrated that both adhesive ligands and MMP-degradable sites were necessary for cells to spread. Furthermore, implantation of VEGF-conjugated

scaffolds into a mouse model of hindlimb ischemia resulted in rapid vascularization of the biomaterial that remained stable for at least 4 weeks [74].

Exciting progress has been made in mimicking the proteolytic recognition of natural ECMs in synthetic polymer gels. Protease-sensitive peptides can be categorized in two main general groups: Plasmin-sensitive peptides and MMP-sensitive peptides. For example, a fibrinogen-derived (R chain, residues $L^{94}I^{119}$) peptide sequence that is combined with RGD cell adhesion site is a plasmin-sensitive peptide. (LR*GD*FSSANNR ↓ DNTYNR ↓ VSEDLRSRI, ↓ indicating the plasmin cleavage site) [75]. Alison et al. evaluated plasmin substrate sites reported in fibrinogen as potential substrate sites in the cross-linker peptide. After considering solubility and hydrophobicity and some other practical parameters, they came up with the engineered peptide sequence CYKNRDC. Because of a negative influence of the aspartate (D) residue on the reactivity of the cysteine © thiol toward vinylsulfone, this residue was eliminated from the final cross-linking peptide design [71].

Sequence (GGGPQG ↓ IWGQGK) is an MMP-sensitive peptide that can be incorporated in the backbone of the PEG block polymers with acrylate terminal groups, which allows cross-linking of precursors into networks. GGGPQG ↓ IWGQGK is a mutated version of α1(I) collagen chain for increased degradation kinetics with various MMPs [76]. Anseth and coworkers incorporated a cysteine-containing bifunctional peptide, CPE ↓ NFFRGD into PEG hydrogels by thiol-acrylate photopolymerization. This peptide has the RGD motif for cell adhesion and the sequence of PENFF for MMP-13-sensitive cleavage [77]. The resulting hydrogels provided a platform that mimics the native upregulation and downregulation of cell adhesive proteins by the cell-secreted enzymes in the ECM for differentiating human mesenchymal stem cells (hMSCs). A review written by Zhu provides more information about other enzyme-sensitive peptides that have been used in the proteolytically degradable PEG hydrogel [78].

ECM Peptides Used in the Hydrogel Backbone to Induce Angiogenesis and Vasculogenesis

Cell adhesion to traditional biomaterials, such as polyethylene, polytetrafluoroethylene or silicone rubber, is based upon nonspecific adsorption of proteins from the body fluids to the material surface. A subset of these adsorbed proteins, including fibronectin, fibrinogen, and vitronectin, promote cell adhesion by interacting with the corresponding adhesion receptors on the cell surface. To achieve similar cell adhesion in a more well-defined synthetic context, several investigators have tested ECM protein-derived cell adhesive peptides as a component of biomaterials. These peptides are based on the primary amino acid sequence or structure of the receptor-binding domains of proteins such as fibronectin and laminin. Early work demonstrated an important possible advantage of working with short adhesion peptides, rather than the complete parent protein, as

the peptides could be displayed in a manner that enhanced peptide availability to cell-surface receptors [63]. Cell adhesive peptides are mainly derived from four ECM proteins, including fibronectin (FN) (e.g., RGD, KQAGDV, REDV and PHSRN), laminin (LN) (e.g., YIGSR, LGTIPG, IKVAV, PDGSR, LRE, LRGDN and IKLLI), collagen (e.g., DGEA and GFOGER), and elastin (e.g., VAPG). RGD is the most commonly used cell adhesive peptide, perhaps due to its long history of use and its effectiveness in promoting cell adhesion. RGD peptides are typically used in either linear (RGD) or cyclic (cRGD) form. Research has demonstrated that cRGD peptides have the advantage of increasing the affinity to integrin $\alpha_v\beta_3$ and enhancing biological activity up to 240 times in comparison with linear RGD analogues [79]. This enhanced effectiveness of cRGD is biomimetic, as the RGD sequence in the cell-binding domain of FN is exposed at the tip of a loop with a spatial constraint that results in increased affinity for cell binding. Thus, incorporation of cRGD peptides into PEGDA hydrogels can better mimic the native RGD loop structure and benefit cell adhesion [80].

In another study, Hubble et al. showed that within the adhesion protein fibronectin, the tetra-peptide REDV is a more specific ligand for integrin receptor $\alpha_4\beta_1$, which is present on the endothelial cell but not the blood platelet. It would be beneficial to use this specificity to develop vascular grafts that support endothelial cell adhesion and migration, while rejecting the adhesion of blood platelets [63].

Hydrogel Stiffness and Porosity

The physical properties of hydrogels can be regulated by the chemistry of the polymeric backbone, its hydrophilicity, polymer concentration, and cross-linking density. Increasing the cross-linking density and/or monomer concentration generally results in increased stiffness and reduced degradation rates because of a larger number of bonds that need to be cleaved during degradation of the material. The stiffness of hydrogels can be adjusted by varying the percentage of polymer used in the solution before the cross-linking procedure and can be controlled by adjusting the cross-linking agent and the cross-linking density during hydrogel formation. Increasing the number of bonds in the polymer also limits the ability of water molecules to diffuse in and out of the material. Thus, the degree of cross-linking of polymer networks can be used to tailor both the structural stability and the porosity of the material [81]. As a result, the degree of cross-linking is an important aspect in regulating the transport of solutes through hydrogel structures [82]. A recent study by West and colleagues showed that hydrogel stiffness affects the degree of endothelial tubule formation. In stiffer hydrogels, tubule formation was reduced and cell clusters remained short and rudimentary, as cells failed to migrate as much as they do in softer matrices [62]. Ghajar et al. studied the effect of matrix density on the regulation of 3D capillary morphogenesis and demonstrated a key role for both matrix stiffness and ligand density [83].

6.4.2.2 Seeding of Vascular or Vascular-Inducing Cells in the Scaffold

Encapsulation of ECs and supporting cells within biomaterials are common strategies that have attracted a great deal of attention. This encapsulation strategy benefits from cell signaling, differentiation, and migration as well as the dynamic interactions between cells that provide the biochemical environment beneficial for the ensuing tissue remodeling. For example, to mimic skin tissue vascularization, Black et al. co-cultured HUVECs, human-derived fibroblasts and keratinocytes (skin cells) housed within a collagen-based scaffold [84]. Biochemical coordination between the ECM generated by the fibroblasts and growth factors such as VEGF and TGF-β secreted by neighboring cells in the presence of HUVECs promoted the development of a vascular network both in vitro and in a mouse model [85, 86]. Furthermore, recent studies highlight the benefits gained from the addition of epithelial cells—those that line the body cavities such the gut and lungs—to stabilize and regulate the size and formation of capillaries within the vascularized model. Thus, various cell types can play complementary roles in tailoring vascularization.

The idea of implanting a co-culture of ECs with ECM-forming fibroblasts or bone-forming osteoblasts in vivo was validated by Alajati et al. HUVECs and osteoblasts were encapsulated within a scaffolding material composed of VEGF and FGF-2 in Matrigel (a murine tumor–derived ECM), fibrin, and thrombin, and the biomaterial was implanted subcutaneously in a severe combined immunodeficiency (SCID) mouse model for up to 20 days. The result was a durable perfused vascular network in vivo [87]. In another study, HUVECs were used to promote the human MSC differentiation into an endothelial lineage, promoting the formation of 3D vascular structures for up to 2 weeks in a Matrigel-based ECM [88]. A mature vascular network was also formed by combining HUVECs and human MSCs in a polymeric scaffold in vivo 4–7 days after implantation, thus accelerating the functional remodeling of the implant when used as a bone graft [89].

All in all, the scaffold is a crucial component that regulates the dynamic vascularization process. Scaffolds used in this area are either natural biodegradable materials, such as collagen and Matrigel, or synthetic biodegradable scaffolds, such as poly-L-lactic acid (PLLA) or poly-D,L-lactic-co-glycolic acid (PLGA). However, because of shortcomings seen in their mechanical strength, durability, immunogenicity, and other application-specific requirements, researchers continue to develop more suitable scaffolds for endothelial-cell-based vascularization for specific applications.

6.4.2.3 Use of Microfabrication Technologies to Engineer Branched Microfluidic Channels Within Biocompatible Materials

Microengineered scaffolds containing channels can be used to seed ECs to form a confluent endothelium on the walls of the vascular channels [90]. Microengineering techniques can be categorized into two different groups based on the

dimensionality of the produced structures: (1) *techniques that produce planar structure* such as photolithography and molding; and (2) *techniques that produce 3D structure* such as direct ink writing and omnidirectional printing. Photolithography is a process that uses light illumination through a mask to generate structures from light-sensitive materials, while molding is a process that uses a hollowed-out pattern to which a deposited material conforms. Photolithography and molding are both planar. A 3D structure can result from stacked 2D structures that comprise channels with rectangular cross sections instead of channels with circular cross sections [6]. Raghavan et al. developed a novel approach to control endothelial tubulogenesis by spatially patterning cells within micromolded collagen gels. ECs cultured within microscale channels that were filled with collagen hydrogel organized into tubes with lumens within 24–48 h of seeding. These tubes extended up to 1 cm in length and exhibited cell–cell junction formation characteristic of early-stage capillary vessels [91]. In another study, a 3D tissue construct composed of endothelialized hollow vascular structures was produced using a self-assembled monolayer (SAM)-based cell deposition technique and a hydrogel photocross-linking method to provide a robust hydrogel-based scaffold for endothelial cell attachment [92].

Direct ink writing and omnidirectional printing within a gel reservoir can create 3D vascular structures in vitro. In a recent study by Chen et al., a printing approach was used to generate a micropatterned sugar-based sacrificial layer around which cell-laden hydrogels could be built. The sugar-based layer was then dissolved, creating a 3D microarchitecture consisting of microvascular networks. Moreover, Chrobak et al. validated the hypothesis that the existent flow and shear conditions within such microscale channels are favorable for endothelium sustainability [58].

Zheng and co-workers created microvessels that replicated some aspects of angiogenesis using silicone molds together with casting gels made out of collagen. The researchers report that the microvessels were lined with continuous endothelium and did not leak. Moreover, when activated with appropriate biochemical signals, the vessels produced new branches and recruited mural cells, which normally associate with blood vessels and affect their functions. As such, the device not only allows the researchers to shape the network, but also permits the biological elements (cells and their products) to reshape or remodel the system dynamically [90]. Despite these substantial advances, new technologies are required to more accurately recreate the complexity of native tissues and enable formation of robust, functional microcapillary networks [93].

6.4.3 Mechanisms for Connecting MicroVessels to MacroVessels

The in vitro formation of mature vessel networks ready to anastomose with the host vasculature shortly after implantation has the potential to dramatically improve the rate of oxygen and nutrient delivery and waste product removal and

thus increases the viability of larger implanted tissues. Rapid (~1 day) anastomosis of engineered vessels with host vasculature is likely necessary for the survival and function of tissue specific cells, especially for oxygen-sensitive cells such as cardiomyocytes, hepatocytes, and various stem cells, all of which are of tremendous interest in the field of regenerative medicine [94]. Limited studies suggest that during embryonic vasculogenesis and angiogenesis, anastomosis is accomplished via connection of extended cellular processes followed by lumen propagation through intracellular and intercellular vacuole fusion, with macrophages playing an accessory role. However, it is not known whether this is the only mechanism for connecting vessels. Without a basic understanding of the cellular mechanisms of anastomosis, it is difficult to develop strategies for accelerating this critical step for perfusing engrafted tissues [95]. This section will describe the different mechanisms that are involved in anastomosis, based on a limited range of insights published to date in this area.

6.4.3.1 Engineered Blood Vessel Networks Connect to Host Vasculature via Wrapping-and-Tapping Anastomosis

Cheng et al. showed that implanted vascular networks anastomose with host vessels through a previously unidentified process of "wrapping and tapping" between the engrafted ECs and the host vasculature. At the host-implant interface, implanted ECs first wrap around nearby host vessels and then cause BM and pericyte reorganization and localized displacement of the underlying host endothelium. In this way, the implanted ECs replace segments of host vessels to divert blood flow to the developing implanted vascular network. The process is facilitated by high levels of matrix metalloproteinase-14 and matrix metalloproteinase-9 expressed by the wrapping ECs. These findings open the door to new strategies for improving perfusion of tissue grafts and may have implications for other physiological and pathological processes involving postnatal vasculogenesis. They found that tip cell connections and vacuole fusion were not integrally involved in host-implant vascular anastomosis, but instead, the engrafted endothelial networks wrapped around host vessels at the host-implant interface and then replaced sections of the underlying vessel wall to tap into the host blood supply [95].

6.4.3.2 Tensional Forces in the Collagen Matrix Control Directional Capillary Sprouting and Anastomosis

Formation of capillary anastomoses is associated with tensional remodeling of the collagen matrix and directional sprouting of outgrowing capillaries toward each other. To analyze whether directional sprouting is dependent on cytokine gradients or on endothelial-cell-derived traction forces transduced through the ECM, Korff and Augustin designed a matrix tension generator that enables the application of defined tensional forces on the ECM. Using this matrix tension generator, causal

evidence is presented that tensional forces on a fibrillar ECM such as type I collagen, but not fibrin, were sufficient to guide directional outgrowth of endothelial cells [96].

6.4.3.3 High Density of Co-transplanted Fibroblasts Promote Rapid Anastomosis of Endothelial Progenitor Cell-Derived Vessels with Host Vasculature

Chen et al. have shown that both endothelial progenitor cell-derived endothelial cells (EPC–ECs) and a high density of fibroblasts significantly accelerate the rate of functional anastomosis, and that pre-vascularizing an engineered tissue may be an effective strategy to enhance transport of nutrients in vivo. In this study, Chen et al. developed three-dimensional engineered vessel networks in vitro by co-culture of ECs and fibroblasts in a fibrin gel for 7 days. Vessels formed by cord blood EPC–ECs in the presence of a high density of fibroblasts created an interconnected tubular network within 4 days, compared with 5–7 days in the presence of a low density of fibroblasts. Vessels derived from human umbilical vein ECs (HUVECs) in vitro showed similar kinetics. Implantation of the pre-vascularized tissues into immune-compromised mice, however, revealed a dramatic difference in the ability of EPC–ECs and HUVECs to form anastomoses with the host vasculature. Vascular beds derived from EPC–ECs were perfused within 1 day of implantation, whereas no HUVEC vessels were perfused at day 1. Further, while almost 90 % of EPC–EC-derived vascular beds were perfused at day 3, only one-third of HUVEC-derived vascular beds were perfused. In both cases, a high density of fibroblasts accelerated anastomosis by 2–3 days [94]. This study and others described above emphasize the critical need to select suitable cell types and engineer surrounding microenvironments that optimize vascular network formation and anastomosis with the host in vivo.

6.5 Challenges for Future Translation of Engineered Tissue Vessels to the Clinic

Balloon angioplasty, stent placement, graft bypass surgery, and use of pharmacological agents are current treatment options for vascular diseases. Vascular grafts that are being used in patients can be divided into three categories, in order of decreasing diameter. Large- and medium-caliber synthetic grafts are used in the thoracic and abdominal cavities with good long-term outcomes. Almost 1,200,000 small-caliber grafts (<6 mm) are used every year for vascular access, to relieve lower limb ischemia and for coronary bypass surgery. Autologous veins or arteries are being used to replace small-caliber arteries, but in 30–40 % of patients these are not available due to prior harvesting or preexisting conditions. Using synthetic

grafts, which provide poor outcomes, is often the only option left for those patients. It is reported that ~50 % of these synthetic grafts will occlude within 5 years, potentially leading to amputation [97].

Considering the need for vascular graft that is been estimated to be about 1.4 million in the US alone [98], there is a need for engineered blood vessels that are nonthrombogenic, withstand adequate burst pressure, show appropriate remodeling responses and are vasoactive [99]. Thus, for successful clinical translation of biomaterials that we discussed in Sect. 6.4, it is essential that researchers identify parameters that can be controlled to promote and regulate angiogenesis and vasculogenesis. The long-term in vivo function of various engineered vascular networks and tissue-engineered vessels still need to be further investigated.

Moreover, it is important that the engineered tissue vessels elicit the least inflammatory response. Ideally, a tissue-engineered vessel should not be immunogenic, nor should it induce thromboembolic complications or excessive and prolonged inflammation. Unfortunately, few biomaterials exist which can be considered biologically inert. For example, Teflon® (expanded polytetrafluoroethylene; ePTFE) and Dacron (polyethylene terephthalate—PET) vascular grafts function well in large-diameter graft applications without endothelial cell coverage but when used in peripheral applications, one half of these grafts occlude within the first five years of implantation [52, 97]. Moreover, preexisting pathology or existing risk factors could affect the long-term success of the implants. For example, implantation of a vascular graft in an atherosclerosis-prone patient results in decreased patency. So designing new approaches that account for the pathological status of the tissue, organ, or patient on the engineered tissue vessel could increase the rate of translational success of tissue-engineered vessels [97, 100].

It is also important to keep in mind efforts to link in vivo with in vitro research successfully in tissue engineering. In vitro culture of tissues and vascular cells provides the basis for our understanding of endothelial cell biology, cell-shape regulation, and blood vessel responses to physical forces. However, most in vitro models lack the three-dimensional complexity, blood flow, cell–cell interactions, and proper extracellular (matrix) environment that are typical of living tissues [101]. In vitro systems that more faithfully mimic the "context" of the native vasculature may lead to more informative in vitro studies.

After an initial period of hype and hope, we are now closer to clinical application. The prospects of using scaffolds, cells, and biochemical or biomechanical stimuli to create functional tissues such as valves and arteries are a power previously unimaginable. However, critical challenges remain for translation of blood vessel tissue engineering strategies. While the field continues to address these challenges, and to further understand the intricate biology of the endothelium, novel biomaterials and cell sources will be critical. In view of emerging advances in biomaterials synthesis/design and stem cell biology, tissue-engineered blood vessels at both the macroscale and the microscale may soon impact thousands of patients in need of tissue regeneration and repair.

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