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Stem cell-derived vasculature: A potent and multidimensional technology for basic research, disease modeling, and tissue engineering

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Abstract

Proper blood vessel networks are necessary for constructing and re-constructing tissues, promoting wound healing, and delivering metabolic necessities throughout the body. Conversely, an understanding of vascular dysfunction has provided insight into the pathogenesis and progression of diseases both common and rare. Recent advances in stem cell-based regenerative medicine – including advances in stem cell technologies and related progress in bioscaffold design and complex tissue engineering – have allowed rapid advances in the field of vascular biology, leading in turn to more advanced modeling of vascular pathophysiology and improved engineering of vascularized tissue constructs. In this review we examine recent advances in the field of stem cell-derived vasculature, providing an overview of stem cell technologies as a source for vascular cell types and then focusing on their use in three primary areas: studies of vascular development and angiogenesis, improved disease modeling, and the engineering of vascularized constructs for tissue-level modeling and cell-based therapies.

Keywords

Stem cell; Pluripotent stem cell; Induced pluripotent stem cell; Embryonic stem cell; Endothelial cells; Vascular; Vasculature; Vascular biology; Smooth muscle; Tissue engineered vasculature; Blood vessels; Stem cell therapy; Tissue engineered blood vessels

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1. Introduction

Being able to move toward the creation of more complex models of disease pathophysiology and tissue-engineered therapies has required advances in our understanding of and ability to replicate blood vessel formation, growth, and support of surrounding tissues. Primitive vascular structures appear early in human development, helping to guide the specification and organization of underlying tissues and defining a wide variety of congenital defects. The process of neoangiogenesis has long fascinated researchers studying diseases as diverse as macular degeneration and arthritis. Indeed, vascular biology has been a primary focus of research into tumor formation, growth and metastasis; inhibitors of vascular processes have been investigated as anti-cancer therapeutics for decades. More recently, the presence of a functional blood supply has been identified as critical to the success of organ and tissue transplantation efforts – including graft survival and rejection – and in the engineering of functional replacements.

With recent advances in regenerative medicine, the field of vascular biology has seen rapid progress. Pluripotent stem cell biology has garnered significant attention in the last two decades, headlined by the establishment of human embryonic stem cell lines (ESCs); the discovery and development of somatic cell “reprogramming” to create induced pluripotent stem cells (iPSCs); and the development of protocols to differentiate pluripotent and multipotent stem cells or directly convert other terminally differentiated cell lineages into vascular precursors. Advances in tissue engineering and biomaterials have led to new scaffolds and strategies for creating three-dimensional (3D) vascular networks and new insights into how the extracellular matrix and surrounding microenvironment contributes to angiogenesis. The concomitant development of increasingly precise techniques for genome editing, including Zinc-finger nucleases, TALENs, and CRISPR-Cas9, opens up the possibility of editing cell lines and animal models to further study vascular development and disease pathogenesis.

In this brief review, we survey recent advances in stem cell biology and tissue engineering as they have been applied to the creation of stem cell-derived vasculature through two main approaches. After presenting advances in pluripotent stem cell-derived vascular modeling, we discuss the ways in which this technology has been: 1) applied to studies of basic developmental biology and vascular pathophysiology; 2) used to model vascular-related disease pathogenesis; and 3) incorporated as a three-dimensional foundation for functionally vascularized, bioengineered tissues. These vascularized tissue approaches will be loosely categorized into those utilizing *in situ* induction (recruiting endogenous vessel formation in tissue engineered grafts) and those which attempt graft pre-vascularization (engineering vessels directly into grafts).

2. Advances in stem cell-derived vascular models

Creating realistic cell-based vascular models requires sources of each of the cellular components of the desired vessels: endothelial cells (ECs), pericytes (perivascular, or support, cells), vascular smooth muscle cells (v-SMCs) appropriate to the desired vessel type, and other tissue-specific cell types that interact with the vasculature (astrocytes in the

central nervous system, for example). A diverse array of stem cell technologies have matured as potential sources for vascular precursors: pluripotent cells [1] such as ESCs [2] and iPSCs [3]; and various types of multipotent (or “adult”) SCs such as mesenchymal stem cells, umbilical cord blood-derived stromal cells, amniotic fluid-derived stem cells, adipose-derived stem cells, and hemangioblasts [4–6]. With these new SC sources, researchers have been able to move beyond primary cell culture and develop lines with particular characteristics, sourced from human patients with particular genetic characteristics or mutations [7]. Vascular cell types can now be generated using stem cell technology through three main pathways: 1) differentiation directly from stem cells obtained from human sources; 2) reprogramming of terminally differentiated cells (often fibroblasts or peripheral blood) through a pluripotent intermediate and then differentiated; or 3) through direct conversion/transdifferentiation from another cell type.

Investigators have been working to develop more robust, efficient, defined, and GMP-compliant (clinically applicable) SC differentiation protocols to generate the necessary vascular cell types for research and eventual therapy (Fig. 1) [2,3,8–13]. The role of individual culture components, culture conditions, biomechanical stimuli, and microenvironmental factors has been elucidated using both standard 2D *in vitro* culture techniques as well as more advanced suspension culture systems, 3D microenvironments, and biomaterials-based approaches [14]. For example, various standard techniques of SC culture have been modified with stimuli to promote early vascular linear specification, as diversely illustrated by the use of nitric oxide to inhibit multipotent vascular stem cell differentiation in two dimensions [15], of TGF- β 1 to induce the formation of tubular structures in ESC embryoid body (pseudo-3D) cultures [16], and biomechanical strain to induce enhanced ECM production in v-SMCs [17].

Meanwhile, investigators have developed biomaterial-based platforms such as micropatterned surfaces [18] and electrospun nanofibrous scaffolds [19], incorporating chemical as well as biomechanical stimuli [20] to improve the efficiency of vascular differentiation and to study cellular responses to these stimuli. Specifics of the exact SC differentiation protocols is beyond the scope of this review and can be found elsewhere [21].

Much of the focus has been on the ECs themselves, and extensive work has gone into studying the molecular pathways governing endothelial specification from pluripotent cells [22]. However, there is also an increasing consensus around the importance of including a robust population of perivascular cells in engineered blood vessel models [23–25]. It has been shown that pericytes and related cells are highly angiogenic, either when used as direct cell therapy *in vivo* or when co-cultured in 3D constructs with ECs that form vascular networks [26].

Alongside advances in vascular stem cell biology have been advances in biomaterials to support stem cell culture, differentiation, and self-organization into functional tissues. Combining cells with scaffolds allows the formation of three-dimensional (3D) culture systems and the development of rudimentary networks of blood vessels. Researchers have applied a wide variety of biodegradable scaffolds [27], both natural and synthetic. The advantage of natural scaffolds is that of biocompatibility. However, synthetic scaffolds are

often more durable, have greater mechanical stability, and are tunable to manipulate cellular behavior and network formation [28–30]. These scaffolds and hydrogels can often be engineered with modified microenvironmental features to enhance the proliferation and self-organization of vascular cells embedded within. For example, locally hypoxic conditions are a particularly attractive feature that investigators have attempted to precisely modulate [31–34] because of the demonstrated ability of controlled hypoxia to enhance vessel formation in 3D tissue-engineered structures *in vitro* [35] and the relevance to the tumor microenvironment in cancer biology.

3. Modeling vascular development, pathophysiology, and disease using stem cell-based and tissue-engineered models

As our abilities to derive vascular cell types and combine them with other technologies continue to grow, so too has our ability to use these components to realistically model human vessel development [36], the vascular niche [37], and vascular remodeling [38]. Using these models, researchers hope to gain insight into processes of angiogenesis and wound healing, to model blood flow dynamics and vessel mechanics under certain conditions, to develop more robust vascular grafting techniques, and to probe the microenvironments necessary for enhanced vessel growth and inform future biomaterial and hydrogel development.

3.1. Modeling vascular development and physiology

Vessel development and maturation are frequently modeled *in vitro* to study the growth of EC vessel- and tube-like structures. Traditional approaches have involved studying primary and stem-cell derived vascular cells in standard 2D tissue culture using matrix coatings and co-culture formulations. More recently, vascular development has been modeled within 3D extracellular biomaterials. Through these rudimentary vascular models, tube morphogenesis can be studied in the presence of cytokines, defined factors, matrix components, and supporting cells in co-culture [39,41]. These models have become more advanced over the past decade, with more complete control of the 3D topographical cues that guide microvascular development [42,43] and the introduction of genetic controls [44]. These approaches have long been used with primary EC cultures; however, with the increased use of iPSC-derived EC, models of network formation, barrier formation, and sprouting have become more robust and easily modifiable [45].

Proliferation of stem cell technologies has allowed more extensive investigation of tissue-specific vascular niches. This knowledge, in turn, has great potential to further inform PSC-derived vascular models especially as they apply to the study (and eventual engineering of) particular tissue types [46]. For example, even within seemingly straightforward populations of ECs and v-SMCs lie subtle diversity in developmental origin yielding unique properties, necessitating the development of protocols to differentiate these subtly different populations (for example, coronary vascular smooth muscle cells as opposed to those found in bladder and aorta [47]). Unique vascular populations have been found to differentiate alongside cardiomyocytes from a common PSC-derived cardiovascular progenitor cell (CPC) precursor [48].

The neurovascular sphere has been studied particularly extensively due to its unique features, its relevance to disease pathogenesis (neuroinflammation, cerebrovascular accident, psychiatric disorders, and more), and its import as a barrier to drug delivery to the CNS. Studies to more fully define the cerebrovascular niche and to characterize cell types will help distinguish different phases of differentiation from stem cells and allow more precise models of vascularized neural tissue [49] and the healthy and diseased blood-brain barrier. The hope is that the use of stem cell-derived vasculature as a basis for early-stage CNS modeling can serve as a paradigm for in vitro developmental process capture. As stem-cell derived neurovascular cell types are purified at all stages of differentiation, researchers have used them to recapitulate neural development in a 3D context [50], and multiple groups have studied the development of the vascular microenvironment from common neural stem cell precursors [51]. The pathways are also now known to go in reverse: in the setting of cerebrovascular ischemia/hypoxia, resident vascular pericytes have demonstrated multipotent stem cell activity [52].

The presence of a blood-brain barrier and the intimate interaction of astrocytes and other additional cell types with the cerebral vasculature has long fascinated investigators interested in vascular biology. Initial protocols have been developed to generate brain-specific EC from PSCs and to cultivate these cells in multicellular cultures [53,55]. Models of the blood-brain barrier fabricated entirely from stem cell sources have shown promise over primary cell culture for advanced cerebrovascular modeling [54,55].

The vascular and hematopoietic stem cell niches have also been found to be intimately connected, as demonstrated by the use of vascular cell types to promote the differentiation, proliferation, and engraftment of PSC-derived hematopoietic cell types [56]. This interaction has been investigated with great interest, as efforts to study the development of and engineer the bone marrow niche will likely involve concomitant modeling of vascular and hematopoietic niches [57].

3.2. Disease modeling using stem-cell derived vascular cells

It has long been known that endogenous vascular SC populations are implicated in a variety of vascular diseases through both innate and acquired dysfunction [58]. Among the most common of these processes are diabetic vasculopathy, arteriovenous malformation, atherosclerosis, calcification, and ectopic tissue deposition within blood vessels [59]. With this knowledge have come efforts to model these disorders using increasingly robust stem-cell derived vascular models. For example, because of the relevance of the DNA damage response to vascular dysfunction in the setting of atherogenesis, vasospasm, and migraines (among others) [60,61], stem cell-derived vascular cells have been used to model genotoxic insults in order to detect changes in multicellular “vascular” responses and functionality [62]. Stem cells with particular genetic characteristics, including from patients with rare diseases, have enabled a deeper mechanistic understanding of rare vascular phenotypes [63]. More advanced 3D cultures have been used to model tumor development and metastasis through intravasation [64,65] and to create a model of perfusable solid tumor for novel drug screening [66].

Organ-on-chip technologies have incorporated stem cell-derived vascular cells and cell types of many other organ systems throughout the body into microfluidic platforms mimicking human circulatory physiology [67,68]. These devices have been demonstrated to have a wide variety of possible applications, including high-throughput stem cell screening in response to chemical and mechanobiological cues, models of drug delivery, and full organ system modeling, both healthy and diseased [68,71]. Vascular-specific diseases such as atherosclerosis, deep vein thrombosis, and pulmonary hypertension have both been successfully modeled using this technique, and these chips have also been used to study the interplay of vascular dysfunction with other organ-level dysfunction in complex diseases of the lung and liver [68].

4. Stem cell-derived vasculature as cell therapy and functional tissue engineering

Vascular tissue engineering first consolidated as a field around the goal of developing vascular graft replacements for individual vessels [72,73]. Researchers used everything from decellularized cadaveric donor vessels to completely synthetic materials such as Dacron and Gore-Tex to more complex mixtures of cells and bio-materials. As the field began attempting smaller diameter grafts, neointimal hyperplasia and thrombus formation bedeviled the transplants and many grafts failed either due to insufficient transport properties or biomechanical failure. As these problems surfaced, so did novel biomaterials and combinations with various stem cell components attempting to solve it [74,76]. Investigators have likewise been creating more advanced tissue-engineered vascular grafts utilizing novel bioreactor/culture systems and stem cell sources, including iPSCs [77,78].

From an initial focus on individual vascular grafts, the field has matured toward the creation of complex vascular networks and vascular “beds” as support structures on which tissue regeneration can occur in vivo and on which engineered tissues can be created in vitro for transplantation [79]. Through the application of insights from early vascular development [36], investigators have developed increasingly complex and successful strategies for engineering vascular networks.

Stem-cell derived vasculature is generally envisioned to have therapeutic applications through three major general approaches: direct cell therapy, in situ induction, and tissue-engineered prevascularization [80,81] (the last of which can be subclassified into biomaterial-directed vs. cell-directed/self-organizing). This review is not meant to be comprehensive of all possible strategies for vascularizing tissue-engineered constructs, only to highlight some major themes; a more comprehensive review can be found elsewhere [82].

4.1. Direct cell-based therapy for in situ tissue vascularization

There is great excitement surrounding the use of SC derived vascular cell types as a direct cell therapy, either transplanted (injected) locally or infused systemically for a variety of diseases, most prominently in the setting of peripheral vascular disease/ critical limb ischemia [83,85]. As a paradigmatic example, ECs and mesenchymal precursor cells generated from iPSCs derived from both healthy and type 1 diabetic human sources were

shown to form functional, competent, and durable vessels in vivo when injected in mouse models [86]. Ophthalmologic and cardiac applications are being heavily investigated as well: iPSC-derived vascular progenitors have demonstrated the ability to regenerate ischemic retinal vasculature, relevant in multiple disease contexts such as diabetic retinopathy and macular degeneration [9], while simultaneous delivery of human pericytes and cardiac stem cells additively improve healing the setting of myocardial infarction [87].

4.2. In situ vascularization

In situ vessel development can be incorporated into tissue engineered constructs by taking inspiration from endogenous repair processes: creating bioactive natural and synthetic scaffold impregnated with growth factors, chemotactic molecules, nano-particles, and sometimes populations of support cells.

The paradigmatic approach has been to engineer bioactive acellular scaffolds to promote endogenous neovascularization capability. Synthetic or naturally-derived scaffolds can act as proangiogenic grafts without any embedded cell sources: through the use of incorporated bioactive molecules (such as factors in the PDGF, VEGF, and SDF families), often under spatial and/or temporal control in the scaffold (chemokine gradients, controlled release nanoparticles, etc.), scaffolds can encourage vascularized neotissue development [88].

Beyond simple acellular scaffolds, there exist multiple strategies for in situ induction. One promising strategy involves the delivery of stromal cells and other types of support cells (either directly or embedded in a scaffold) to the site requiring neovascularization. Multiple cell lineages can exert paracrine effects encouraging vascular, with a biomaterial scaffold and then implanting the cell-seeded scaffold. This allows vessels to form and self-organize utilizing both resident and transplanted cells after implantation [89]. In a way, this approach can be considered a hybrid of in situ and prevascularization, as stem cell-derived populations other than the vascular cells themselves are pre-embedded. Vascularized adipose tissue was generated in this manner using stromal-vascular fraction-derived adipose cells embedded in precultivated fibrin gels for soft tissue substitution [90]. A similar strategy has been applied to evaluate the use of both adipose-derived stem cells co-cultured with EC [91] and amniotic-fluid derived stem cells [92] as sources of vascular and perivascular cells for in situ capillary network formation.

Complex platforms have been developed to modulate the paracrine effects of embedded stem cells through customized, self-assembled matrices [93] and by engineering pro-angiogenic factor release capability into the embedded cell populations themselves [94]. One demonstration of this is through the transduction of HIF-1 α into MSCs embedded into gelatin sponges for bone tissue engineering, yielding enhanced transcription of pro-angiogenic gene programs and dramatically improved blood vessel formation within implanted tissue engineered bone [95].

4.3. Pre-vascularization

Pre-vascularization can be scaffold-directed, with vessel channels preformed and then seeded with cells of interest, or cell-directed, where stem cell-derived vascular cells are embedded within the scaffold and allowed to self-organize into tube-like structures. Once a

method is chosen, researchers have a great deal of flexibility over how cells are applied to the scaffolds (embedded vs. seeded using various methods [96]) and how growth within these scaffolds can be controlled.

One of the canonical strategies for prevascularization of engineered tissues is perfusion decellularization, a technique that involves antegrade perfusion of complex tissues with detergents to remove all cellular components and leave behind the endogenous extracellular matrix scaffold including the channels formed by blood vessels in the tissue [97]. This technique first demonstrated downstream applicability through tissue-engineered vessel grafts formed from seeding of PSC-derived endothelial and/or smooth muscle cells onto a decellularized vessel scaffold in a bioreactor, creating fully cellularized vessels whose cell sources can be patient- and application-specific [98]. This strategy has matured beyond individual vessel grafts, however; it has been applied to the creation of decellularized vascular beds within whole tissues, allowing pre-seeding of vascular networks within complex scaffolds for whole-organ tissue engineering [99,101].

While decellularization has received much attention for the presence of ready-made, physiologic channels within these constructs, other methods of creating “scaffold-directed” prevascularized constructs have gained favor as well, including 3D bioprinting [102,104], the use of “sandwich” cell-accumulation cultures [105], and micropatterning [106]. A high profile illustration of biomaterial-defined pre-vascularization using biomaterial-based microchannel networks came with the demonstration that filamentous carbohydrate networks could be printed and precisely manipulated to generate cylindrical vessel-like networks [107]. Once seeded with endothelial cells, these networks allowed perfusion with blood under high-pressure pulsatile flow and supported hepatocytes in large tissue-engineered constructs, overcoming an obstacle that has bedeviled solid organ tissue engineering: hypoxia and necrosis in the core of non-perfused constructs [107]. A similar strategy has been used in hollow channel-modified porous silk scaffolds [108,109].

To highlight a more “cell-directed” approach, research groups have successfully generated functional, 3D vascular networks by embedding stem cells, vascular progenitors, and stem cell-derived terminally-differentiated cells in a diverse array of biomaterial-based hydrogels and scaffolds (Fig. 2) [30,110]. The power of this technique is that these cell populations can all be sourced from the same stem cell precursor and, given the right microenvironmental cues and support structures, cell populations can self-organize according to their own developmental programs into multicellular, functional microvessel networks. Such networks derived from hPSCs have been shown to be fully mature, integrate with host vasculature, and establish blood flow within animal models.

Engineered tissue pre-vascularization has received considerable attention in the fields of hepatic and cardiac tissue engineering in particular. Mixtures of primary and stem cell-derived lines of both vascular and tissue-specific phenotype (e.g. fetal liver cells mixed with umbilical vein endothelial cells and mesenchymal stem cells) have been successfully embedded in 3D scaffolds and allowed to form rudimentary vascular networks among maturing and proliferating hepatic cells, forming engineered pre-vascularized human hepatic tissue [111]. Investigators have also created prevascularized cardiac tissue constructs using

multiple different strategies [81,112,115]. Sheets of entirely iPSC-derived cardiac tissue with both cardiomyocytes and vascular cells have been created and found suitable for transplantation into rat infarcted hearts and were found to successfully engraft and induce neovascularization [116]. Investigators have also created 3D cardiac tissues using stem cell sources and porous scaffolds in advanced cell culture and bioreactor systems [117], and have used vessel explants surrounded by engineered microenvironments to stimulate capillary network growth as a vascularized scaffold [118,119].

5. Future directions

Disease modeling using stem cell-derived 3D models is still in its relative infancy. However, with the advent of patient-derived iPSCs as well as novel genome editing techniques, one can expect these models to yield insights about areas as diverse as connective tissue diseases, autoimmune vasculitis, neurodegenerative disease, cardiomyopathy, muscular dystrophy, even hematopoietic diseases such as hemoglobinopathies and leukemias. All of these have a suspected vascular phenotype and thus a role to play for vascularized models of disease.

As stem cell-derived vascular models continue to expand, so too will their application to create increasingly complex tissue-engineered constructs. Following the model of perfusion decellularization leads us to believe that engineered lung, pancreas, muscle, and skin will soon be layered atop vascularized scaffolds, improving outcomes in tissue engineering. Extension of vascular tissue engineering strategies to the lymphatic system to address such problems as lymphedema and lymph-mediated immunologic dysfunction has also been contemplated [120], and investigators have begun to discuss the use of vascular stem cells in conjunction with tissue engineered models of neural tissue and the bone marrow niche.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

SC	stem cell
PSC	pluripotent stem cell
iPSC	induced pluripotent stem cell
ESC	embryonic stem cell
v-SMC	vascular smooth muscle cell
EC	endothelial cell

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Fig. 1.

(A–H) Spontaneous vascular differentiation in embryoid bodies (EBs). Confocal microscopy of stained 10-15-day-old human EBs. (A) CD34⁺ cells forming vascular networks along the EBs ($\times 200$). (B) Projection of CD34⁺ cells serial sections within an EB ($\times 600$). (C) Occasionally round cells are stained with CD34⁺ ($\times 600$). (D and E) CD31⁺ cell groups organized in specific channel-like structures ($\times 600$). (F) Elongated smooth muscle actin (SMA)⁺ cells surrounding voids with no detectable network formation ($\times 200$). (G) SMA⁺ and (H) smooth muscle myosin heavy chain (SM-MHC)⁺ cells surrounding voids, forming

flat thick tubes ($\times 600$). (I–M) Sprouting from differentiated human embryonic stem cells. Differentiated cells were allowed to aggregate for 24 h in medium supplemented with human vascular endothelial growth factor (VEGF₁₆₅), after which they were seeded into type-I collagen or Matrigel in the same medium. (A) Tube-like formation after 7 days in type-I collagen gel and (B) aggregate sprouting in Matrigel (low and high magnification). (C) Two different areas of 3-dimensional sprouting on Matrigel, documented with Hoffman microscopy. (D) Longitudinal histological sections showed (i) seeded cells (*arrow*) penetrating into the Matrigel (M) and (ii) forming primitive tube-like structures (*arrow*) (higher magnification). (E) Transverse histologic sections of the cell-seeded Matrigel exhibited different sizes and shapes of tube-like structures formed within the Matrigel (Scale bars: 100 μm .). [Both adapted from Gerecht-Nir et. al. *Lab Invest* 2003; 83:1811–1820].

Fig. 2.

(A–F) Self-assembly of EVCs to multicellular networks in a 3D matrix. (A) Network formation from BC1 (iPSC-derived)-EVCs in collagen (i) and Hyaluronic Acid (HA) hydrogels (ii). (B) Sorted VEcad⁺ and VEcad⁻ cells encapsulated within collagen gels were unable to form networks. (Insert) Example of a cell with typical stellate morphology, with phalloidin in green and nuclei in blue (Scale bars: 100 μ m.) (C) Vacuole formation was observed after one day as evidenced by light microscopy (LM) (i) and confocal images (ii) of vacuole vital stain, FM4-64, in red and nuclei in blue (Scale bar: 10 μ m.) (D) On day 2,

network formation with enlarged lumen (*i* and *ii*) and cell sprouting (*iii* and *iv*) were visualized by LM images (*i* and *iii*) and confocal images (*ii* and *iv*) of FM4-64 in red and nuclei in blue. (Scale bars: 10 μm in *i* and *iii*; 20 μm in *ii*; 50 μm in *iv*.) (*E*) On day 3, complex networks were observed with enlarged and open lumen, as indicated by confocal z-stacks and orthogonal sections of FM4-64 in red and nuclei in blue (Scale bar: 20 μm .) (*F*) After 3 d, multilayered structures were also detected, as demonstrated by a 3D projection image of NG2 (green), phalloidin (red), and nuclei (blue) showing NG2⁺ pericytes integrated onto hollow structures. Images shown are typical of the independent experiment (Scale bars: 50 μm .) [Adapted from 110]. (G–I) Vascular tube morphogenesis by ECFCs within Hypoxia Inducible (HI) hydrogels. (*G*) Light micrographic images of ECFCs encapsulated within HI hydrogels during 3 days of culture (Scale bar: 50 μm .) (*H*) Confocal microscopic images of ECFCs encapsulated within nonhypoxic gel and hypoxic gel; (*I*) confocal z-stacks and orthogonal sections show lumen formation (indicated by arrows) within the vascular networks (phalloidin in green; nuclei in blue) (Scale bars: 50 μm .) [Adapted from 32]. (J–M) ECFC vascular network growth and complexity in HA hydrogels. (*J*) Left panel: Growth of comprehensive vascular networks at day 3 are demonstrated using LM images at low magnification. Right panel: (*i* and *ii*) are high magnifications of white boxes (arrows indicate branched and elongated vascular networks) (Scale bars: 100 μm .) (*K*) Confocal analysis of vacuole vital stain FM4-64 (cyan; nuclei in blue) demonstrating large lumen within the networks, and (*L*) demonstrated using orthogonal view (indicated by arrowhead) (Scale bars: 50 μm .) (*M*) TEM high-resolution images show cross-sections of matured vascular tube networks with enlarged lumens (Scale bars: 20 μm .) [Adapted from Hanjaya-Putra et. al. *Blood* 2011; 118(3):804–15. doi: 10.1182/blood-2010-12-327338.]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)