Chinese Medical Journal®

Advances in vascularized organoids

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Abstract

The development of organoid technology has led to notable advances in regenerative medicine, disease modeling, and drug screening. However, given the pivotal role of vascular systems in growth maintenance and nutrient delivery, the lack of functional vascular networks within organoids limits the scalability and physiological functionality of these organoids. Efforts to explore strategies for organoid vascularization, such as co-culture, transcription factor induction, microfluidic technology, and the application of biomaterial scaffolds, are underway. These methods have shown distinct advantages across different types of organoids, partially addressing vascularization challenges. However, issues such as limited vascular network integration and insufficient functional maturity remain unresolved. Future efforts should prioritize the development of multi-tiered vascular networks, the application of smart biomaterials, and the creation of personalized vascularized organoids. This review summarizes the advances in this field and explores the potential translation of vascularized organoids into clinical practice, offering recommendations for further research.

Keywords: Vascularized organoids; Co-culture; 3D bioprinting; Microfluidic technology

Introduction

Organoids are complex, organ-like structures generated *in vitro* by cultivating and inducing the proliferation and differentiation of stem cells, resulting in self-organizing formations. In comparison with traditional animal models and two-dimensional cell cultures, organoids mimic the three-dimensional (3D) architecture of *in vivo* organs more accurately, thereby offering a platform with enhanced physiological relevance.[1,2] As a result, organoids show applicability across diverse research fields, including developmental biology, disease modeling, drug discovery, and regenerative medicine.[3–5] Generic cell types, such as vascular endothelial, stromal, and immune cells, have recently garnered substantial attention for their essential roles in various physiological processes.[6] These cells contribute to the formation of the tissue microenvironment by establishing organ-specific phenotypes. To replicate the interactions, structural characteristics, and physiological functions within organs, researchers have focused on developing complex organoids that incorporate multiple cell types, matrix components, and physiological features.

Vascularization plays a crucial role in organoid culture by facilitating oxygen delivery, nutrient transport, and metabolic waste removal, which directly influence organoid viability.[7–9] The diffusion limit for oxygen and nutrients in mammalian tissue is approximately 100–200 μm, which is sufficient for smaller organoids. However, when the organoid size exceeds this threshold, the core regions often undergo necrosis due to hypoxia and nutrient deficiency, leading to compromised functionality.[10] Therefore, achieving vascularization of organoids is crucial for practical applications.

This review comprehensively explores recent advances in organoid vascularization by elaborating on the complex structure of the vascular system, with emphasis on pericyte–endothelial cell crosstalk and the regulatory roles of biophysical cues including fluid shear stress and the extracellular matrix in vascular network formation. It summarizes the significance and developmental progression of organoid vascularization, as well as current *in vitro* strategies for engineering vascularized organoids and their inherent limitations, and proposes an integrated biological–engineering approach incorporating intelligent strategies to establish more stable and efficient vascularization methods, offering new perspectives for organoid-based basic research and clinical applications.

Overview of Complex Organoids

Organoid research has progressed remarkably over the past decade since the advent of induced pluripotent stem cells (iPSCs). In comparison with traditional two-dimensional

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000004136

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Chinese Medical Journal 2026;139(16)

Received: 03-12-2025; **Online:** 10-06-2026 **Edited by:** Yanjie Yin

cell cultures, organoids can replicate the structural complexity of real organs more effectively, establishing themselves as a critical bridge between conventional cell lines and *in vivo* models.[11,12] However, the vast majority of organoids lack organ-specific vasculature, neurons, and immune cells, limiting their potential for modeling complex pathophysiological phenotypes.[13] Recent studies have focused on developing complex organoid models that incorporate vascular endothelial cells, peripheral neurons, and immune cells with organ-specific phenotypes. Xenotransplantation, directed differentiation, and multicellular co-culture are the primary approaches for generating organoids with greater cellular diversity and functional complexity.[14,15] Vascularization of organoids is a critical challenge because the absence of a vascular network restricts organoid growth and factor exchange, thereby compromising nutrient distribution and clearance of metabolic waste.[16] By integrating bioprinting and co-culture techniques, researchers have successfully constructed vascularized complex organoids, including those that mimic the heart, liver, and brain. These advancements have laid a critical foundation for disease mechanism studies and regenerative medicine.[17–19] However, these models face substantial challenges in terms of functional maturity and stability. Future research efforts should focus on optimizing organoid vascularization and innervation to better mimic *in vivo* environments and further explore their potential applications in transplantation and personalized medicine.

Limitations of avascular organoids

Organoids are 3D cellular assemblies derived from stem or progenitor cells cultured *in vitro* that are capable of partially recapitulating the cellular diversity, tissue architecture, and functional characteristics of native organs [Supplementary Figure 1, http://links.lww.com/CM9/C908].[20,21] By modulating culture conditions and signaling pathways, organoids can be directed to form a variety of tissue types, providing powerful models for studying organ development, disease mechanisms, and drug responses.[22–24] Owing to their physiological relevance and scalability, organoids have considerable potential for applications in basic research, personalized medicine, and regenerative therapy.[25] Nevertheless, most organoids lack intrinsic vascularization, which constrains their size, maturation, and functional capacity, often leading to hypoxia and cell death in the central regions.[1] Blood vessels are critical for delivering oxygen and nutrients and play a key regulatory role in organ homeostasis and function.[26] Although recent efforts have sought to reproduce vasculogenesis and angiogenesis within organoid systems, mimicking the structural complexity and physiological functionality of native vascular networks remains a major challenge. Consequently, insufficient vascularization is a critical barrier to fundamental organoid research and translational applications.[1,27]

The construction of mature, perfusable vascular networks *in vitro* offers substantial potential to drive transformative advancements in tissue transplantation technologies and address the therapeutic needs of diseases for which effective treatments are currently lacking.[28–32] Notable progress has been made in the field of *in vitro* vascular reconstruction, particularly in regenerative medicine and organoid culture. Researchers have gradually achieved vascularization in tissue engineering using methods such as co-culture techniques,[33] 3D bioprinting,[34] and microfluidic chip technology.[35] These vascularized organoids have shown immense potential for a wide range of applications [Supplementary Figure 2, http://links.lww.com/CM9/C908].

Vascularized organoids

The establishment of a hierarchical vascular network capable of supporting transplantation, tissue stability, and blood perfusion is crucial for advancing organoid research.[36,37] In comparison with conventional organoids, vascularized organoids offer notable advantages across multiple dimensions [Table 1]. However, the challenges associated with establishing such organoids have long limited the scalability and complexity of constructing clinically relevant tissues.[38,39] In addition to facilitating nutrient transport and diffusion, the vascular network plays a critical role in regulating tissue formation.[40,41]

Table 1: Differences between organoids and vascularized organoids.

Items	Organoids	Vascularized organoids
Tissue structure	Lack vascular networks and show limited internal material exchange.[7]	Contain functional vascular networks, which enhance structural fidelity and support greater volume and complexity.[52]
Functional simulation	Partially reproduce organ function, lack vascular systems, and show limited microenvironment and intercellular interactions.[53]	Show enhanced nutrient supply and waste clearance through vascular networks, thereby approaching physiological functionality and microenvironment.[54]
Cultivation methods	Formed by cultures of adult stem cells or pluripotent stem cells, organoids are mainly dependent on the process of spontaneous organization.[55]	Formed by the introduction of vascular-associated cells through co-culture, 3D bioprinting, and microfluidic chip technologies, resulting in increased technical complexity.[56–58]
Transplant adaptability	Show reliance on host vascularization post-transplantation and present challenges in sustaining long-term survival.[59]	Possess pre-integrated vascular networks that enhance post-transplantation integration and stability.[60]

3D: Three dimensional.

Studies have shown that vascularized tissue grafts demonstrate improved clinical outcomes in terms of innervation and functional recovery. Non-vascularized tissues, when constructed *in vitro*, are limited by diffusion constraints for nutrient supply and cannot survive if the tissue size exceeds 1 mm.[42] Consequently, most tissue-engineering studies are conducted at scales that are substantially smaller than those required for clinical applications.[43] With advancing research, vascularized organoids can use blood vessels to deliver adequate nutrients, promote organoid development, and efficiently remove metabolic waste. In addition, factors secreted by vascular endothelial cells have a profound influence on the physiological functions of organoids. The specific functions of the vasculature are summarized below.

(1) *Substance transport*: Limited mass transport is a critical factor that constrains organoid development and functional maturation. The effective diffusion distance of oxygen, nutrients, and metabolic waste within tissues is only approximately 100–200 μm;[44] thus, non-vascularized organoids depend solely on passive diffusion for exchange. As the size of these organoids increases, the efficiency of oxygen and nutrient delivery decreases sharply and metabolic byproducts accumulate, causing nutrient depletion, metabolic imbalance, and hypoxic necrosis in the central regions. Moreover, diffusion-induced concentration gradients cause heterogeneous cell proliferation and differentiation, compromising the overall structural integrity and functional stability of the organoids.[45] The incorporation of vascularization can establish efficient channels for the transport of gases, nutrients, and metabolites, thereby overcoming the limitations of organoid size and complexity. Consequently, vascularization is essential to generate physiologically relevant organoids with improved viability, maturation, and functional performance.

(2) *Regulatory role of endothelial secreted factors:* Endothelial cells play crucial regulatory roles in organoid formation, maturation, and functional maintenance by secreting diverse angiocrine factors.[46] These factors, including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), angiopoietin, hepatocyte growth factor (HGF), and insulin-like growth factor (IGF), promote angiogenesis, cell proliferation, and differentiation, thereby enhancing nutrient and oxygen delivery and mitigating hypoxia-induced necrosis within the organoids.[47] In addition to driving vascularization, angiocrine factors modulate the fate of epithelial and parenchymal cells through paracrine signaling, thereby promoting tissue-specific functional maturation. For example, HGF enhances the metabolic activity of liver organoids,[48] whereas Notch and Wnt ligands, together with exosome-derived signaling molecules, facilitate intercellular communication and ECM remodeling, thereby increasing the structural complexity and physiological relevance.[49] Collectively, angiocrine signaling is fundamental for overcoming the limitations of organoid vascularization, enabling the generation of highly functional tissue models and enhancing their translational potential in regenerative medicine and disease modeling.

(3) *Demand for specialized organoids:* In certain specialized applications or in the construction of organoids with specific structures, such as the blood–brain barrier,[50] microfluidic organ-on-a-chip systems, and multi-organ chips with circulation,[51] the formation of a vascular endothelium or blood vessels is essential. Similarly, the formation of blood vessels is critical in highly vascularized tissues, such as the liver, kidneys, lungs, spleen, heart, pancreas, and thyroid.

Strategies for Vascularization of Organoids

Currently, organoid vascularization is primarily accomplished through strategies such as endothelial cell co-culture, transcription factor induction, vascularized scaffold integration, 3D bioprinting, microfluidic perfusion systems, and *in vitro* co-differentiation. These methodologies have enabled the successful generation of vascularized organoids across multiple tissue types, including the brain, liver, kidney, heart, intestine, and bone, thereby enhancing nutrient exchange, promoting tissue maturation, and improving the overall functional performance [Table 2].

Co-culture methods

One of the most widely used strategies for organoid vascularization involves co-culturing with endothelial cells [Supplementary Figure 3, http://links.lww.com/CM9/C908]. Supplementation with angiogenic factors such as VEGF and basic fibroblast growth factor (bFGF) can effectively promote endothelial cell migration, proliferation, and neovascular formation. Human umbilical vein endothelial cells (HUVECs) are the most commonly used endothelial source for such co-cultures and have been

Table 2: Vascularized organoids and their construction strategies.

Organoid type	Vascularization strategies
Liver organoids	- Embedded vascularization[61] - Microfluidic technology[62] - Co-culture approach[63]
Lung organoids	- Microfluidic technology[64] - Self-assembly approach[65] - Bioprinting technology[66]
Kidney organoids	- Bioprinting technology[67] - Induced vascularization[68]
Intestinal organoids	- Self-assembly approach[69] - Induced vascularization[65] - Co-culture approach[70]
Neural organoids	- Bioprinting technology[71] - Self-assembly approach[18]
Skin organoids	- Bioprinting technology[72] - Tissue-engineered scaffold method[73]
Bone organoids	- Co-culture approach[74] - Bioprinting technology[75]
Cardiac organoids	- Microfluidic technology[64] - Co-culture approach[76] - Induced vascularization[77]

successfully integrated into liver, brain, kidney, pancreas, intestine, cardiac, and pulmonary organoids, enabling the establishment of functional vascular networks that enhance organoid maturation and physiological relevance.[56,58,78–81]HUVECs can form vessel-like structures surrounding organoids, thereby enhancing oxygen and nutrient exchange, which is critical for sustaining tissue viability. Shi *et al*[82] co-cultured brain organoids with HUVECs, inducing their differentiation into brain microvascular endothelial-like cells and generating vascularized brain organoids capable of long-term *in vitro* maintenance with functional perfusion. A growing body of evidence has indicated that organ-specific endothelial cells promote the development and maturation of their corresponding organoids more effectively. For example, liver sinusoidal endothelial cells (LSECs) have been shown to enhance both the structural organization and functional maturation of hepato-biliary organoids.[83] In addition to their use in stem cell-derived models, co-culture strategies have been used to achieve vascularization in several adult stem cell-derived organoids. Nevertheless, in most HUVEC-based co-culture systems, vascularization is confined to the periphery of organoids because endothelial sprouts rarely penetrate the central regions. Consequently, hypoxia and vascular instability persist within the core, largely because of the inhibitory microenvironment that restricts endothelial proliferation, integration, and long-term functionality.[84]

In addition to endothelial cells, vascular smooth muscle cells, pericytes, macrophages, and immune cells cooperate in the formation and stabilization of vascular networks.[85] The use of microvascular fragments (MVFs), which preserve intact vascular lumens and native cellular architecture, has recently emerged as an effective strategy for enhancing vascularization.[86] Yasaman *et al*[87] integrated human embryonic stem cell (hESC)-derived pancreatic cells with MVFs to generate vascularized islet organoids. After subcutaneous transplantation, these organoids promoted the differentiation of pancreatic progenitors into β-cell clusters, restored normoglycemia in diabetic mice, and markedly improved cell survival. However, despite their structural similarity to native vasculature, the MVF-derived vessels exhibited limited perfusion and nutrient-exchange capacity, constraining their long-term tissue stability. Wrublewsky *et al*[74] further demonstrated that adipose-derived MVFs can spontaneously assemble into stable microvascular networks when co-cultured with osteoblasts and rapidly vascularize after subcutaneous implantation, highlighting their potential as critical building blocks for integrating angiogenesis with osteogenesis in bone tissue engineering. Similarly, Lê *et al*[88] established patient-derived tumoroids from resected lung adenocarcinoma tissues and co-cultured them with adipose-derived MVFs and autologous peripheral blood mononuclear cells (PBMCs). This approach could successfully induce vascularization and reconstruct the immune microenvironment. The vascularized patient-derived tumoroids obtained by this approach exhibited extensive vascular networks and immune cell infiltration, substantially enhancing their physiological relevance and providing a robust platform for anti-angiogenic drug screening and therapeutic evaluation.

With the continued advancements in organoid technology and the successful generation of 3D vascular organoids,[89] their co-culture with other tissue organoids has received increasing attention. Li *et al*[90] replaced HUVECs with iPSC-derived endothelial and mesenchymal cells to generate fully humanized liver buds. Ahn *et al*[91] co-cultured 3D vascular organoids with cortical brain organoids, creating brain constructs that contained both neural networks and vascular architectures and exhibited hallmark features of the blood–brain barrier. Similarly, Sun *et al*[18] simultaneously generated 3D vascular and brain organoids from hESCs and successfully established functional vascular networks that were tightly integrated with neural tissues.

The existing approaches to achieve organoid vascularization predominantly rely on co-culture and co-differentiation–based self-assembly strategies. However, these approaches are generally restricted to generating microvascular networks of approximately 15–50 μm in diameter and remain insufficient for establishing a fully mature vascular system.[92] Challenges such as heterogeneous vessel distribution, limited functional maturation, technical complexities, and high experimental costs hinder large-scale production and clinical translation.[23] Future studies should integrate emerging technologies, including growth factor induction and 3D bioprinting, to develop structurally stable and functionally competent vascularized organoids, thereby advancing their applicability in tissue engineering and regenerative medicine.

Induction of the transcription factor ETV2

Regulating the activity of certain key transcription factors may enhance the efficiency of mesenchymal differentiation into endothelial cell lineages. The Ets family of transcription factors, particularly ETV2, has been shown to play a critical role in hematopoiesis and vasculogenesis. Increasing evidence suggests that ETV2, either alone or in combination with other transcription factors, can reprogram fibroblasts or mesenchymal stem cells (MSCs) into endothelial cells.[93–95] Morita *et al*[96] demonstrated that ETV2 can directly convert primary adult human skin fibroblasts into functional vascular endothelial cells (ETVECs). ETV2 synergizes with endogenous FOXC2 in fibroblasts to induce the expression of functional endothelial molecules, including EGFL7 and vascular endothelial factors. *In vitro*, ETVECs exhibit endothelial cell characteristics, and upon transplantation into NOD SCID mice after encapsulation in Matrigel for 28 days, they form mature, functional blood vessels. Furthermore, ETVECs have been shown to significantly improve blood flow recovery in a hindlimb-ischemia model in *BALB/c-nu* mice. Wang *et al*[97] developed an endothelial cell generation protocol by first differentiating iPSCs into mesodermal progenitor cells, which was followed by the delivery of *ETV2*-encoding mRNA, efficiently differentiating 13 different iPSC lines into endothelial cells. This approach addressed the inefficiency of standard differentiation methods that rely on endogenous ETV2. However, this method had some limitations, such as the induction of only a low degree of vascularization and the absence of the ability to generate a complete vascular lineage (e.g., MSCs).[95] Although ETV2, as a proangiogenic

transcription factor, shows substantial potential in organoid and tissue engineering, it still faces limitations in terms of timing control, functional specificity, long-term maintenance, and clinical translation. Additional research may be needed to combine it with other methods or further optimize ETV2 application strategies to enhance its effectiveness and stability in vascularization.

Vascularized scaffolds

The construction of *in vitro* tissues containing vascular networks using biomanufacturing technology has become a major focus of domestic and international research [Supplementary Figure 4, http://links.lww.com/CM9/C908]. In this regard, most of the existing research has primarily focused on designing and fabricating three-dimensional scaffolds using biocompatible materials that support cell adhesion, proliferation, and differentiation, ultimately forming structures that resemble natural vascular networks. By guiding vascular formation, vascularized scaffolds can enhance the survival and functionality of transplanted tissues or organs, particularly in large-scale tissue engineering, where they address challenges related to oxygen and nutrient transport. However, natural biomaterials present certain limitations in vascularized tissue engineering. For instance, collagen has poor mechanical properties, and fibronectin is both costly and exhibits excessive mechanical stress, making both unsuitable for vascular network formation.[98] The existing research efforts have largely focused on improving and combining natural biomaterials or exploring new natural biomaterials to enhance vascularization in engineered tissues.[99–102] Quinlan *et al*[103] used bioactive glass/collagen-glycosaminoglycan scaffolds for bone tissue engineering. *In vitro* experiments showed that when the bioactive glass particles had a diameter of 100 μm, the scaffolds significantly increased VEGF secretion by endothelial cells and promoted the formation of tubular structures. Vascularized scaffolds require good biocompatibility and degradability. However, identifying materials that can support cell growth and degrade harmlessly *in vivo* remains challenging. If the material degrades too quickly or too slowly, it may compromise the scaffold function and tissue repair. In addition, the manufacturing process for vascularized scaffolds is complex and costly, presenting a major challenge in achieving large-scale production and standardization while maintaining scaffold quality and functionality.

3D bioprinting

3D bioprinting has profound implications for fields such as medicine, biological research, tissue engineering, and drug development.[104,105] 3D bioprinting technology can be used to create complex microvascular networks, which are crucial for building functional tissues and organs. By precisely controlling the deposition of bioinks, researchers can print structures resembling the natural vasculature, which support cell growth and tissue maintenance. This technology can enable the creation of biocompatible three-dimensional structures by stacking specific bioprinting materials layer-by-layer, offering new possibilities for constructing vascularized organoids.[106,107] 3D bioprinting technology can enable precise and personalized customization of target tissues or organ scaffolds with vascular channels, facilitating the vascularization of engineered tissues. For example, Li *et al*[108] used 3D bioprinting to construct calcium phosphate bone-cement composite scaffolds with mesoporous silica, featuring a specific pore structure that optimized the release of silicon ions to promote early-stage vascular ingrowth after implantation. Ma *et al*[109] designed a strontium silicate particle-based bioink and used extrusion printing to deposit suspensions of endothelial and fibroblast cells onto layers of this bioink, producing a multicellular scaffold that mimicked vascularized skin. Leveraging the proangiogenic properties of strontium silicate, this scaffold significantly enhanced skin vascularization and wound healing. In one study on cardiac tissue engineering,[110] the researchers developed an embedded 3D bioprinting technique that deposits endothelial cells, smooth muscle cells, and matrix materials at precise locations to create myocardial tissues with layered, branched, and multilayered vascular networks. By embedding a monolayer of fused endothelial cells within the lumen formed by connecting three matrix components, the researchers successfully constructed biomimetic vessels. These vessels embedded within the cardiac tissue supported fluid flow, enabling the maturation of cardiac organoids under perfusion and thereby exhibiting spontaneous beating and effective responses to pharmacological treatments. In another study,[75] researchers used aspiration-assisted bioprinting (AAB) to fabricate 3D bone-mimetic tissue constructs using MSCs and HUVECs as bioinks. The two cell types were co-cultured in a 1:1 ratio and preconditioned through a 10-day osteogenic induction period before printing, which enhanced their osteogenic potential and improved the structural integrity of the printed constructs. This technological advancement paved the way for scalable biomanufacturing of vascularized, organ-specific tissues for drug testing, disease modeling, and therapeutic applications.

Microfluidic technology

Microfluidic chips, also known as biochips, represent an interdisciplinary technology designed for the precise manipulation of fluids at the microscale.[111] Organoid-on-a-chip technology has allowed the integration of organoid culture processes and experimental functions onto solid-phase materials such as silicon, glass, plastic, or metal. This system allows precise control of fluid dynamics and simulates complex biological environments, thereby enhancing the functional capabilities of conventional organoid models.[112,113] A vascularized organoid system based on microfluidic technology can meet the demand for endothelial cells in perfusion cultures, enhance the secretion and release of various cytokines, and simulate blood flow characteristics at varying flow rates. This approach more closely replicates the functional dynamics observed in real organ tissues. Yu *et al*[114] designed a microfluidic chip with a microvascular network to culture endothelial cells and enhance their angiogenesis. These chips simulated realistic hemodynamic conditions and provided an optimal environment for studying angiogenesis *in vitro*. Shaji *et al*[115] used microfluidic chips to precisely position

endothelial cells, enabling the construction of more complex vascular networks. They developed a co-culture system of cerebral organoids (COs) and vascular beds on microfluidic chips using a combination of culture conditions (NDM/EGM2) to support co-differentiation and vascular network formation. Within this environment, the neuronal lineage of the COs was preserved, and the preformed vascular beds maintained their structural integrity for at least 10 days. As the endothelial cells migrated, they formed vascular connections through budding structures, ultimately resulting in tissue vascularization. Salmon *et al*[116] achieved the co-development and interaction between organoids and vascular cells using a microfluidic chip. On this chip, pericytes and endothelial cells derived from human pluripotent stem cells (hPSCs) self-assembled into a vascular network and interacted with COs, ultimately forming vascularized neural organoids. Rajasekar *et al*[26] developed a microfluidic platform called IFlowPlate, which facilitated the co-culture of patient-derived colon organoids with self-assembled vascular networks by optimizing the ECM composition and culture medium formulations. This platform enabled parallel *in vitro* generation of up to 128 perfusable and vascularized colon spheroids, offering a robust tool for drug screening and disease modeling based on vascularized organoids. Similarly, Du *et al*[117] used microfluidic chip technology to establish a co-culture system consisting of patient-derived tumor organoids, endothelial cells, and fibroblasts, or stromal cells, successfully generating tumor-specific microvascular networks *in vitro*. Patient-derived samples exhibited distinct vascular induction and migration behaviors, which were strongly correlated with clinical metastatic potential and prognostic outcomes. This model can serve as a promising platform for evaluating anti-angiogenic therapies and investigating tumor–vasculature interactions. The advent of microarray manufacturing technology offers a new pathway for large-scale production of vascularized organoids. Microfluidic technology, with its advantages of precise control, dynamic regulation, high throughput, and three-dimensional structural assembly, has become a vital tool for promoting tissue vascularization.[51] These benefits can not only enhance the experimental efficiency but also open new possibilities for investigating more complex vascularization processes.

Co-development

Recent research on vascularized organoids has progressed from the simple incorporation of vascular networks to the development of complex systems driven by coordinated self-organization of multiple cell lineages [Supplementary Figure 5, http://links.lww.com/CM9/C908]. Although traditional endoderm-derived organoids, such as those representing the intestine, lungs, pancreas, and liver, have achieved substantial advances in morphology and functionality, their structural maturity, cellular diversity, and functional completeness (including secretion, metabolism, and gas exchange) remain inferior to those of native organs. In contrast, during *in vivo* organogenesis, dynamic interactions between the mesoderm (which consists of stromal and endothelial components) and endoderm, along with the concurrent formation of the vascular system, are essential for establishing organ-specific architecture and functional maturation.[118,119] Building on this developmental principle, multilineage co-differentiation has emerged as a key strategy for generating vascularized organoids. Using iPSCs to simultaneously differentiate into endodermal and mesodermal lineages, researchers have successfully reconstructed a range of physiologically functional vascularized organoids *in vitro*.[120,121] This approach enhances vasculogenic capacity while promoting the spatial organization and functional integration of epithelial and stromal components, thereby substantially improving the physiological relevance and translational potential of these models. Miao *et al*[65] developed an iPSC-based co-induction system that enabled the simultaneous differentiation of endodermal and mesodermal lineages within a single 3D spheroid. By modulating the signaling intensity of bone morphogenetic protein, they precisely regulated the ratio of epithelial to endothelial/mesenchymal progenitors, generating a well-coordinated multilineage structure. This approach successfully produced lung and intestinal organoids with distinct organ-specific functions. Notably, the lung organoids developed alveolus-like architectures exhibiting gas-exchange and barrier properties. Transplantation studies revealed that the vascular networks within these organoids integrated with the host vasculature and became perfusable, thereby enhancing tissue maturation and cellular differentiation. Furthermore, the model could recapitulate the phenotypes of developmental diseases, such as the disrupted epithelial–endothelial interactions caused by FOXF1 deficiency, demonstrating its potential as a platform for studying developmental disorders and genetic disease mechanisms. Sang *et al*[122] applied a multilineage co-differentiation strategy to generate pancreatic organoids. By inducing hPSCs to simultaneously differentiate into mesodermal and endodermal lineages and modulating Wnt signaling while supplementing VEGF alpha during the early stage (days 0–3), researchers promoted the formation of ECs. The resulting culture contained a mixed population of approximately 13.9% ECs while maintaining high expression of key pancreatic progenitor (PP) markers, including PDX1 and NKX6.1. Transcriptomic analysis revealed that vascularized pancreatic progenitors exhibited enhanced metabolic activity and upregulated expression of endothelial and mesenchymal marker genes, indicating greater structural and functional maturation. Upon further differentiation, the vascularized pancreatic progenitors developed into β-like cells capable of insulin secretion and physiological glucose responsiveness, providing a promising cellular source for regenerative therapies in diabetes. In summary, the mesoderm–endoderm co-differentiation strategy based on pluripotent stem cells enabled synchronized generation of vasculature and epithelium within organ primordia, establishing a foundation for constructing vascularized organoids with perfusion capacity and physiological functionality. This approach has progressed from the exploratory stage to achieve substantive outcomes, with organoid models expanding from the initial intestinal and lung systems to the pancreas, liver, and other organs. Notably, major advances have been made in structural maturation, cellular diversity, and functional performance, including secretion, metabolism, and lineage-specific differentiation.

Challenges Associated with Vascularized Organoids and Future Directions

The vascularization of organoids is a critical step in organoid culture because it enhances their similarity to real organs, improves their functionality, and simulates authentic microenvironments. This step is essential for studying organ development, disease mechanisms, and for *in vitro* drug screening. Furthermore, vascularized organoids offer substantial potential in clinical transplantation since they can integrate more rapidly with the recipient's circulatory system. However, despite the notable progress in organoid vascularization research in recent years, several key challenges remain to be addressed.

Cell types and organ specificity

The cell types involved in vascularization are complex and exhibit organ-specific characteristics. HUVECs are commonly used in vascularization modeling because of their ease of handling and low cost; however, the vasculature formed by HUVECs lacks the specificity required to support organ-specific structures, limiting the depth of mechanistic studies. Endothelial cells from different organs vary in terms of subcellular structure, angiogenic potential, endocrine functions, and vascular regulatory capabilities, underscoring the need for approaches tailored to organ-specific vascularization.[123–128] For example, endothelial cells in the brain are tightly joined to maintain the blood–brain barrier, whereas glomerular endothelial cells form a semipermeable membrane to enable filtration.[129] In the liver, spleen, and bone marrow, endothelial cells have larger intercellular gaps that facilitate the exchange of macromolecules and blood cells.[130] In addition, the interactions of endothelial and surrounding cells are critical for maintaining vascular homeostasis, and the composition and functions of these supporting cells display substantial organ-specific characteristics.[131] Therefore, understanding the perfusion data of different organs and the distribution of their arterial, venous, and capillary networks is the primary step for constructing vascularized organoids.

Complexity of angiogenesis mechanisms

Replicating the *in vivo* microenvironment for *in vitro* vascular growth and development remains challenging. This difficulty arises primarily because angiogenesis depends on various biochemical factors, such as VEGF, angiopoietins, PDGF, transforming growth factor β, and FGF, as well as physical factors, including shear stress and pressure from blood flow.[128,132–134] Recent studies have demonstrated that endothelial cell metabolism plays a crucial role in angiogenesis, supplying energy for vascular sprouting through glycolysis while also regulating signal transduction, gene expression, and transmembrane transport functions.[135,136] As a result, simulating the microenvironment for *in vivo* vascular growth remains a major challenge in research on organoid vascularization. Natural hydrogels have been widely used as culture matrices because of their biocompatibility. By adjusting the stiffness, porosity, and bioactive components of these hydrogels, the biochemical signals and metabolic microenvironments essential for angiogenesis can be delivered.[137,138] Integrating engineering technologies to provide tunable biological and physical stimuli holds promise for improved construction of functional vascular systems.

Discrepancies between vascularized organoids and native organs

Vascularized organoids and native organs still show substantial discrepancies in terms of vascular complexity and functionality. Vascular formation in native organs is an inherently complex and ongoing process that persists throughout an organism's life. In contrast, organoid vascularization primarily relies on external interventions such as the addition of specific growth factors or endothelial cells. Consequently, the maturity of the resulting vascular networks requires improvement, and both the size and density of these networks differ considerably from those of the natural vasculature. Despite the advances in 3D printing and microfluidic technologies for constructing vascularized organoids, the existing methodologies still face challenges in accurately replicating the coordinated interactions among different blood vessel types. Issues persist in achieving mechanical stability of vascular networks, responsiveness to hemodynamic forces, and effective interactions with other cell types.[106,134,139] Advancements in biomaterials, bioengineering technologies, and stem cell biology, combined with multi-level vascularization strategies, are expected to enable functional vascularization across various types of organoids.

Reproducibility and stability of organoid culture techniques

The existing research on organoid vascularization is largely limited to the experimental stage. Variations in technical approaches, culture systems, and experimental conditions among studies have led to inconsistent vascularization outcomes. Even in studies using identical methods, differences in the organoid size, cellular composition, and matrix properties can result in fluctuations in vascular formation.[140] As organoids increase in structural complexity, cellular heterogeneity, and culture duration, the issues related to reproducibility and stability in vascularization become more pronounced. Advancements in 3D culture systems have substantially improved the structural integrity and physiological relevance of organoid models. Micro-organoid systems derived from tissues such as the mammary gland and lungs have demonstrated enhanced structural stability and functional maturation, allowing more accurate modeling of complex physiological and pathological processes.[141–143] The mechanical properties of the ECM are the key determinants of organoid development. Matrix stiffness and viscoelasticity regulate cell spreading, polarization, and lineage specification: excessively rigid matrices restrict cellular expansion and differentiation, whereas overly soft matrices lead to structural collapse. In contrast, matrices with moderate and dynamically tunable stiffnesses provide physiologically relevant mechanical cues that promote organ-specific differentiation and functional organization. Thus, ECM mechanics can serve as a physical scaffold as well as a

critical regulator of cell fate and tissue morphogenesis.[144–146] In summary, rational design and dynamic modulation of the mechanical properties of the ECM can enhance organoid structural complexity, physiological functionality, and experimental reproducibility. With continued optimization of culture technologies, the development of stable, reproducible, and vascularized organoid systems will lay a robust foundation for applications in regenerative medicine and disease modeling.

Conclusions

Organoids have emerged as well-established research models, with vascularization substantially enhancing their resemblance to native organs and improving their functional and microenvironmental simulations. These advancements are crucial for studying organ development and disease mechanisms, and for *in vitro* drug screening. Vascularized organoids can also provide a physiological foundation for *in vivo* transplantation, facilitating revascularization and ensuring long-term survival post-implantation. The existing approaches for the construction of vascularized organoids using co-culture systems and bioprinting technologies have several limitations, such as the immature and simplistic vascular networks obtained by these approaches as well as the high technical barriers associated with 3D printing and microfluidic systems, which require specialized equipment and expertise. Researchers have explored iPSC-based direct induction methods to generate vascularized organoids, aiming to produce models with uniform cell sources and phenotypes that are closer to those of native organs. However, these approaches remain in the early experimental stages, and the key to achieving functional vascularization lies in accurately identifying and quantifying the differences between *in vivo* and *in vitro* environments and incorporating these parameters into vascularization protocols, a highly challenging endeavor. The integration of advanced 3D bioprinting technologies offers the potential to construct *in vitro* external circulation systems that can interface with organoid-derived vascular networks. Moreover, leveraging artificial intelligence-driven automation for intelligent and real-time control of internal and external circulatory and metabolic systems could pave the way for the development of mature vascular networks within organoids. Such breakthroughs will substantially accelerate progress in pharmaceutical research and industrial applications.

Funding

This study was supported by grants from the National Key R&D Program of China grant (No. 2022YFA1105602), and the 1.3.5 Project for Disciplines of Excellence, West China Hospital, Sichuan University (No. ZYGD23023).

Conflicts of interest

None.

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How to cite this article: Shen LL, Ma JH, Wei X, Shi G, Deng HX. Advances in vascularized organoids. Chin Med J 2026;139:2409–2419. doi: 10.1097/CM9.0000000000004136