REVIEW

Angiorganoid: vitalizing the organoid with blood vessels

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Abstract

The emergence of the organoid simulates the native organs and this mini organ offers an excellent platform for probing multicellular interaction, disease modeling and drug discovery. Blood vessels constitute the instructive vascular niche which is indispensable for organ development, function and regeneration. Therefore, it is expected that the introduction of infiltrated blood vessels into the organoid might further pump vitality and credibility into the system. While the field is emerging and growing with new concepts and methodologies, this review aims at presenting various sources of vascular ingredients for constructing vascularized organoids and the paired methodology including de- and recellularization, bioprinting and microfluidics. Representative vascular organoids corresponding to specific tissues are also summarized and discussed to elaborate on the next generation of organoid development.

Keywords

▶ blood vessels
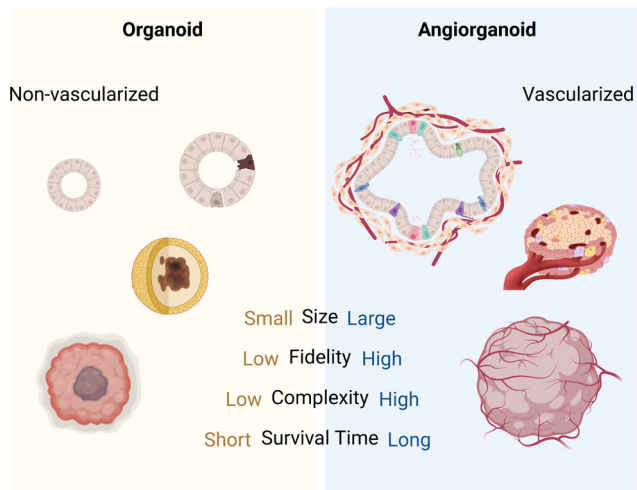▶ angiorganoid
▶ organoid
▶ bioengineering

Introduction

Organoids should comprise at least two cell types which further organize into a 3D structure recapitulating some of the key features of the native organ (1). Although organoids have gained significant attention in the academic community and industries, there remain major challenges that prevent such models from achieving a broader application. One of the key limitations is that when an organoid reaches a certain size and complexity, central necrosis is inevitable unless vascularization is achieved. During organ development, blood vessels usually grow simultaneously to not only nourish but also remodel and reshape the organs. Lack of vascularization limits the size, complexity, maturation and function of organoids. Emerging technologies from vascular biology, synthetic biology and bioengineering are being combined for nourishing the organoids with vasculature. The rapid progression of such interdisciplinary talk leads to the 'angiorganoid' in which the organoids are upgraded with infiltrating blood vessels. Angiorganoid resembles the native vascularized organs more than the traditional organoids and opens the door for probing the coupling between blood vessels and tissues *in vitro* (Fig. 1).

To be vascularized or not?

Perfusion is usually achieved when transplanting the angiorganoid in an immune-compromised mouse model

Figure 1
From organoid to angiorganoid: blood vessels matter. When organoids grow beyond a certain size, oxygen and nutrients are difficult to diffuse evenly throughout the organoids and the internal cells undergo apoptosis due to hypoxia. Due to the limited size and complexity, the functions of organoids are also compromised. Compared with organoids, angiorganoids could grow not only larger and longer but also have more complex structures which are more akin to real organs (2, 3, 4).

in vivo, the host blood vessels anastomose with the existing blood vessels in the organoid to achieve the perfusion. However, studies showed that even non-perfused blood vessel networks already benefit the maturation and long-term function of organoids (Fig. 2).

Transfer of metabolic materials and wastes

The diffusion of oxygen, nutrients and metabolic wastes in tissues is limited, generally allowing cells to survive within 100–200 μm (5) which limits the size and complexity of non-vascularized tissues or organoids. Vascularization is the key to prolonging the survival time of organoids grown beyond the diffusion limit of oxygen. Besides, the diffusion of oxygen and nutrients will produce concentration gradients, resulting in non-uniform cell proliferation and differentiation and affecting the overall function and maturity of organoids (6).

Organ-specific angiocrine factors

Blood vessels in different organs have specific endothelial cells (ECs), which can be classified as barrier-forming continuous ECs, fenestrated ECs and sinusoidal ECs, as well as two specialized types of Schlemm's canal and high endothelial venules (7). The organotypic ECs have common and specific functions. Besides barrier effect, ECs

can also secrete a variety of angiocrine factors, including secreted and membrane-bound inhibitory and stimulatory growth factors, cytokines, chemokines, ECM components, exosomes and other cellular products that modulate organ homeostasis and regeneration (8).

Organ-specific ECs adjust proliferation, self-renewal and differentiation of stem and progenitor cells. In the presence of ECs, Notch and Hes1 are activated to stimulate the self-renewal of neural stem cells (9). Brain-derived nerve growth factor, pigment epithelium-derived factor, neurotrophin-3, placental growth factor-2, betacellulin and other paracrine factors, as well as membrane-bound proteins ephrin B2 and Jagged 1, are involved in regulating the homeostasis and regeneration of NSCs (8). Liver sinusoidal ECs secrete hepatocyte growth factor (HGF) to orchestrate liver regeneration (10). ECs can also regulate metabolism through paracrine. EC-derived EGF-like 7 promotes pancreatic progenitor self-renewal and impairs further differentiation into hormone-expressing cells (11).

Instructive mechanical cues

The flow of blood in vessels will generate mechanical forces, including a variety of fluid shear stress (FSS). The types and dimensions of FSS have an impact on the morphology, function, proliferation, gene expression and even lesions of ECs and then indirectly affect other tissues and cells. Laminar shear stress (LSS) or steady unidirectional shear stress (USS) has anti-inflammatory and antiatherosclerotic effects (12), while oscillatory shear stress (OSS) produced by a disturbed flow can induce inflammation and oxidative stress in ECs (13).

Vascular ECs have multiple mechanosensors, such as plexin D1 (PLXND1) and syndecan 1 (SDC1) (14, 15), and have several signal pathways involved in the response to FSS. Krüppel like factor 2 (KLF2) is a transcription factor inducible by USS and mainly expressed in ECs, which mediates the inhibition of the BMP/SMAD1/5 pathway (16) and induces the upregulation of the miR-143/145 (17) to inhibit atherosclerosis. Moreover, by modulating the integrin–Gα13–RhoA pathway, USS inhibits YAP/TAZ to achieve atheroprotective effects (18). Alk5-Shc pathway regulates endothelial–mesenchymal transition in response to FSS (19).

In addition to the shear forces, mechanical forces of perfusion also include the mechanical stretching of ECs caused by vasodilation. Lorenz *et al.* found that blood perfusion leads to mechanotransduction in hepatic endothelium inducing the production of HGF (20).
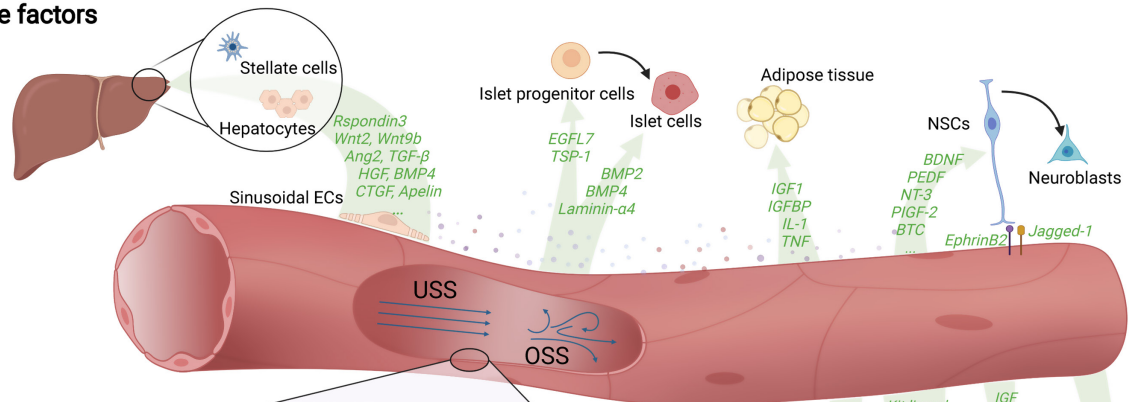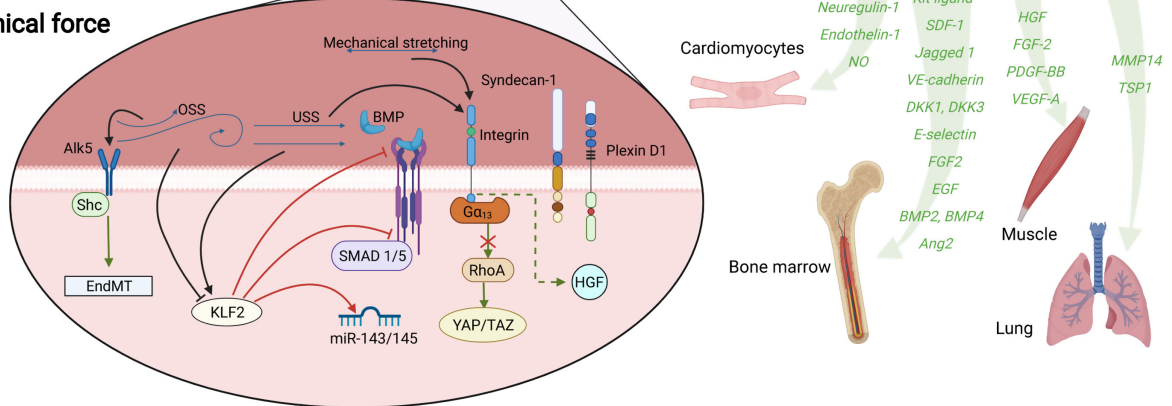
Figure 2

Effects of perfusion and angiocrine factors on organoid parenchymal cells. (A) Organ-specific vascular ECs can secrete a variety of organ-specific angiocrine factors to regulate organ homeostasis, regeneration and metabolism by paracrine or juxtacrine. (B) FSS affects the morphology and function of ECs in many ways and then indirectly affects other cells. Several mechanosensor, such as plexin D, syndecan-1, Alk5 and integrin, can respond to mechanical force. By regulating the related signal pathways, OSS can cause atherosclerosis and EndMT, while USS induces antiatherosclerotic effects. Mechanical force may influence the secretion of ECs, for instance, stretching can increase HGF secretion.

Vascular ingredients for angiorganoid

Vascular cells including ECs and perivascular cells are two important components that constitute the blood vessels. To achieve vascularization in organoids, basic building blocks of vessels need to be constructed. In the following sections, we will discuss some approaches to vascular cell production including co-differentiation and transcription factor overexpression and introduction of exogenous vascular cells in different tissues or organoids (Fig. 3).

Co-differentiation

Vascular cells share the same origin of mesoderm with organs such as kidneys and hearts, which makes the co-differentiation of organoids and vascular tissues possible. During the kidney organoid differentiation process, Low *et al.* discovered a population of nephron progenitor cells (NPCs) with the potential to develop into mature ECs using flow cytometry. Moreover, by increasing glomerulus-

to-tubule ratio using γ-secretase inhibitor DAPT which impedes the generation of proximal tubules, Low *et al.* were capable of encouraging vascular endothelial growth factor (VEGF-A) secretion, which then promotes the *in vitro* vascular network generation by stimulating epithelial cell growth (21). The study carried out by van den Berg *et al.* also indicated the presence of human-induced pluripotent stem cell (hiPSC)-derived vascular cells in the kidney organoids without any exogenous VEGF (22). While improvements have been made in generating vascular cells along with kidney organoids, vascular cells alone without anastomosis are not sufficient in supporting kidney functions, especially in organs such as kidneys that rely heavily on their circulation system. Further research is needed for elucidating the process of constructing perfusable blood vessels with co-differentiated vascular cells (23).

Since hearts are mesoderm layer-derived organs as well, the organ-vascular cell co-differentiation approach used on kidneys can also be applied to cardiac tissues. The protocol of Lewis-Israeli *et al.* utilized Wnt pathway
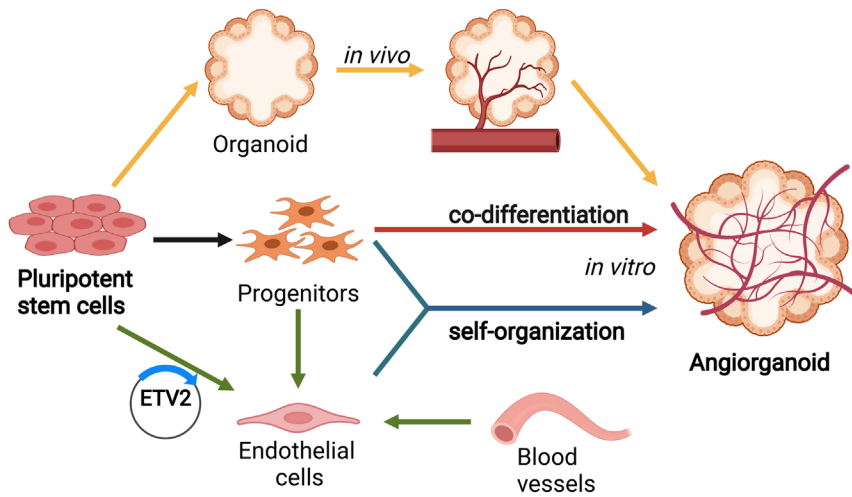
Figure 3
Vascular components of angiorganoid. One of the most common strategies to build angiorganoid is to introduce exogenous vascular cells harvested from real blood vessels. Those vascular cells self-organized into vascular networks and merged with organoids through either wrapping or penetration. Secondly, certain tissue-specific organoids (e.g. heart, kidney) share the same progenitors with vascular cells, adding extra EC-inducing cytokines (e.g. VEGFA) would render the vascular network to co-develop with other parenchymal cells to complete the vascularization. Thirdly, since ETV2 is a decisive factor of endothelial fate, forward programming of pluripotent stem cells (PSCs) directly into ECs through transient activation of ETV2, regardless of the exogenous factors, is highly efficient and desirable. Lastly, the *in vivo* environment could function as an incubator to inoculate the blood vessel.

modulation to generate heart-like organoids with normal structure, function and vasculature. During the cardiac differentiation process, ECs were found attaching to the myocardial tissues, which indicates the initiation of vascularization *in vitro*. In addition, cardiac organoids treated with growth factors BMP4 and activin A showed an increasing number of ECs generated from pluripotent stem cells, which then can induce further vessel growth (24). The research by Hofbauer *et al.* also fortifies the feasibility of generating vascular cells and cardioids simultaneously by comparing ECs in cardioids with differentiated ECs from other sources. The Smart-seq2 analysis showed that cardioid ECs were most consistent with ECs from vascular organoids generated by Wimmer *et al.* (25). Furthermore, Hofbauer *et al.* also discovered that low Wnt along with low activin A could induce EC differentiation and development, which might help optimize vascularization *in vitro* in the future.

Interestingly, researchers found that ECs emerged even during the differentiation of endoderm-derived organs such as intestines and livers. Although vascular cells are generally considered mesoderm derivatives, the research of Goldman *et al.* proposed the possibility of generating functional endoderm-derived ECs during liver organoid differentiation. They identified CD31+ ECs originated from FOXA2+KDR+ fetal hepatoblast progenitor cells, which suggested that mesoderm might not be the only source of ECs but also the endoderm (26). Additionally, Holloway *et al.* discovered that ECs emerged at a very early stage of human intestinal organoid (HIO) differentiation that gradually disappeared over time. To preserve the EC population, Holloway *et al.* performed RNA-seq to identify key molecules and introduced survival factors to enhance the production and durability of ECs (27). While discovering endoderm layer-derived ECs is quite intriguing, more strict lineage tracing data have to be presented in the future to show that a single bipotent progenitor could indeed give rise to both endoderm and ECs. After all, it is possible that the small portion of the contaminated mesoderm progenitors could still yield vascular cells.

Synthetic vasculature driven by ETV2

Activation of endothelium-inducing transcription factors can promote the generation of ECs. Wang *et al.* developed a novel hiPSC-derived human induced EC (hiECs) differentiation protocol, where they delivered chemically modified ETV2 mRNA to mesodermal progenitor cells
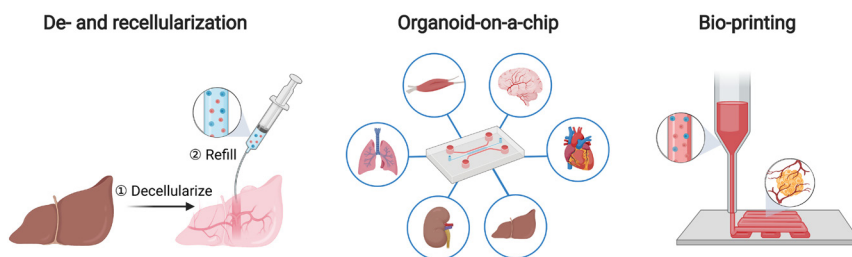

Figure 4
Bioengineering angiorganoid strategies include de- and recellularization, organoid-on-a-chip and bio-printing.

(hMPCs). This approach achieved consistency among 13 different hiPSC lines with very high efficiency (>90%) in hiECs generation. Importantly, the hiPSCs-derived ECs were capable of forming perfused blood vessels upon transplantation, which provides a great opportunity for studying vasculature–organ interaction in a deeper manner (28). Cakir *et al.* used human embryonic stem cells (hESCs) that carried an inducible ETV2 circuit to encourage endothelial-like cell formation in a neurogenic medium. The ETV2-induced endothelium successfully generated perfusable vascular networks in brain organoids (2).

Self-organization with exogenous vascular cells

Vascularization can be achieved by co-culturing PSCs with exogenous vascular cells as well. One way of inducing vascularization is by co-culturing tissue-specific organoids with human umbilical vein ECs (HUVECs) and other supportive cell types. Takebe *et al.* incorporated hiPSC-derived hepatic cells with HUVECs and human mesenchymal stem/stromal cells (hMSCs), a type of multipotent stem cell that can give rise to diverse cell types including fat cells and muscle cells. The vascular system built with HUVECs became functional after joining the host vasculature which renders the vascularized liver buds (LBs) more resembling the adult liver (29). HUVECs co-culturing is not limited to hepatic tissues. Takahashi *et al.* attempted to incorporate HUVECs and hMSCs along with a variety of tissues including pancreatic islets, brain fragments, heart fragments, intestine fragments, kidney fragments, liver fragments and lung fragments (30). Instead of using differentiated tissues, Shi *et al.* co-cultured HUVECs with hiPSCs or hESCs. They discovered that during the differentiation of hiPSCs and hESCs into cerebral organoids, HUVECs gradually gained characteristics resembling ECs in brain tissues. The cerebral tissue-specific EC development might better assist angiogenesis in the brain (31). As an alternative to HUVECs, organ-specific ECs might generate vasculature more native to the organ environment. Yap *et al.* incorporated mouse liver progenitor cells (LPCs) with isolated mouse liver sinusoidal ECs (LSECs) to achieve vascularization in hepatobiliary organoids. Compared to LPC/HUVEC organoids, LPC/LSEC developed more native hepatic structures with liver-specific vascular networks, which demonstrated the marked effect of LSECs on hepatobiliary organoids development and the feasibility of applying this idea of using native ECs on other organs (32). Another way of obtaining ECs is by differentiation from pluripotent stem cells. Pham *et al.* co-cultured iPSCs-derived ECs with brain organoids in Matrigel, and ECs-covered organoids successfully developed vascular systems *in vivo* (33).

While HUVECs and iPSC-derived ECs might seem like a perfect solution to obtain vascular cells, perivascular cell generation cannot be directly achieved using this approach. The protocol designed by Wörsdörfer *et al.* integrated MPCs into organoids. When treated with platelet-derived growth factor or VEGF, MPCs can grow into smooth muscle cells (SMC) or ECs respectively, which serve as critical building blocks of vascular walls. Electron microscopy also indicated the presence of periendothelial cells connecting to the endothelium in a natural manner. The finger-like processes in the connection site might be crucial for interactions and communications between ECs and other perivascular cells (34).

Other than single vascular cells, microvascular fragments (MVF) isolated from adipose tissue can serve as an optimal choice of vascular tissue source. Nalbach *et al.* integrated pancreatic islet cells with MVF composed of functional arterioles, venules and capillaries. MVF still keeps the features of native microvessels such as containing abundant ECs covered by SMCs and secreting angiogenic factors. The incorporation of MVF with pancreatic islet cells then initiated prevascularization *in vitro*, which greatly improved vascularization after transplantation (35).

The emergence of a variety of methods for generating vascular cells makes efficient organoid vascularization possible. Although researchers have already made huge progress in the area, further study is still waiting for us. For example, due to the difference in characteristics of different types of organoids, some methods of vascular cell generation might be more suitable than others, which makes finding the optimal way of inducing vascular cell generation in specific organoids an important future direction. Moreover, compared to producing ECs, the study of generating perivascular cells is still falling behind and needs further research. At last, researchers also need to optimize the current protocol to enhance the efficiency of vascular cell genesis.

Host-derived blood vessels

We have already learned about how to build pre-constructed vascular networks, and there are other important methods such as developing post-engraftment vascularization under the guidance of the host organ.

Transplanted organoids are engrafted into host organs where the existing vascular network can assist

the development of the vasculature in organoids. The progress and situation of vascularization can be visualized via lectin labeling, immunofluorescence and graft whole-mount imaging (36). With the host-assisted engraftment technology growing gradually more mature, attempts have even been made in complex organs such as brains. Mansour *et al.* (37) developed an *in vivo* model where brain organoids derived from human pluripotent stem cells are engrafted into the mouse brain. Excitingly, not only vascular networks in organoids were successfully developed but also axonal growth was found in the transplanted brain organoids which have the ability to respond to environmental stimuli. Even though the brain organoid's vascularization does not require the presence of ECs, organoids coated with ECs show an increase in vascular plexus growth (37). While researchers are currently unable to confirm the capability of brain organoids in damaged brain tissue restorement, this approach can lead to a promising future in post-damage cell treatment of brains.

Other than organoid transplantation in brains, organoid transplantation under the kidney capsule is probably the one that attracts the most attention. Since the kidney itself is highly vascularized, a large number of pre-existing blood vessels in the host might greatly assist the vasculogenesis in organoids (22). The research of van den Berg (22) achieved glomerular, tubular and vascular maturation after kidney organoid engraftment without the assistance of any vascular growth factors. Moreover, the vascular systems developed in the kidney organoids after transplantation are connected to those in the host kidney via anastomosis. With further improvement using results from this study, organoid transplantation in kidney capsules might serve as a solution to organoids vascularization, especially for those with high requests for oxygen and nutrients. In addition to the kidney capsule, epididymal fat pad (EFP) could also serve as an implant site for organoids, similar to the human counterpart of the omentum (38). Compared to the kidney capsule, EFP site is less invasive while providing a favorable vascularizing niche that warrants further exploration (39).

Bioengineering solutions for angiorganoid

De- and re-cellularization

One way to bioengineer the vascular system in organoids is called the de- and re-cellularization method, which utilizes blood vessel skeletons uprooted from organs (Fig. 4). With cellular parts carefully removed by gentle detergent, extracellular matrix (ECM) and the complete

vascular system are preserved. Isolated vessel networks can serve as a bioscaffold with ECs and tissue-specific cells were reseeded through the vascular system to support stable vascular system functioning. Researchers have utilized macrovascular networks separated from the whole liver organ, while ECM molecules were preserved to provide a 'more authentic' environment for cell maintenance and development (40). ECs (e.g. HUVECs) were seeded through previously retrieved vena cava and the portal vein due to the importance of EC in maintaining steady blood flow and normal vascular function. The presence of ECs in main vessels and capillary veins indicates the validity of this method. While no solid evidence confirms that ECs can be evenly distributed throughout the vascular channel due to limited microscopy resolution, a notable decrease in platelet aggregation suggests that ECs reseeding via vascular transportation is relatively successful, but further experiments on EC detection in vessels are certainly needed to ensure sufficient EC re-cellularization.

Additionally, Takeishi *et al.* planted hiPSC-derived ECs along with hiPSC hepatocytes and hiPSC biliary epithelial cells on the decellularized liver serving as a bio-frame. HiPSC-derived vascular ECs (hiPSC-VECs) seeded in the decellularized liver scaffold exhibited angiogenesis with functions resembling actual human livers (41).

Organoid-on-a-chip

Another way of supporting organoid development and preventing oxygen and nutrients shortage is to 'flatten' the organoid itself, which means culturing organoids on microfluidic chips. Organ-on-a-chip model was first designed to replicate and investigate interactions between organs (40). Microchannels on the chip offer transportation of drugs, growth factors and nutrients that can support and/or affect downstream tissues or organs (41). In tumor-on-a-chip research, drugs and nutrients are delivered through microvascular channels to study the effect of different drugs and nutrition resources on tumor progression (42). For retina-on-a-chip research, well-controlled vascular flow and the perfusion system with a constant supply of nutrients and factors provide an optimal environment for the growth of tissue-specific cells (43).

An essential feature of this system is the microfluidic network made of polydimethylsiloxane which plays an important role in organoid monitor and control and multi-tissue/organ connection and communication (41). Biosensors of biomarkers and mini-microscopes were engineered into the chip system, which is capable of non-

stoppingly detecting any changes in secreted proteins running between organoids and observing the condition of organs (44). This technology has been applied to many other research projects. Shirure *et al.* (42) utilized the microfluidic platform to monitor tumor progression by tracking cell growth, vascularization and migration. In the study of building retina-on-a-chip, researchers were able to ensure the stability of the system environment by monitoring generated proteins that are crucial for the development of retinal organoids (43). For a more comprehensive summary of this area, an excellent review paper has just been published (45).

Bioprinting

In recent years, unprecedented attempts have been made in 3D bioprinting of angiorganoid to mimic the structure and function of native organs with the vasculature. Possible solutions range from additive manufacturing techniques such as fused deposition modeling (FDM), digital micro-mirror (DMD) method and two-photon-based photopolymerization to substructural methods by tissue removal including sacrificial networks and subtractive fabrication performed by laser (42). While every method has its own advantages and limitations, the diversity of approaches and the combinations of different techniques might be the answer to producing long-lasting organoids.

In contrast to FDM, photopolymerization of cell-harboring gel with a projecting template underneath gives the DMD method a much higher resolution. The 3D structure of the vascular model is constructed by layer-by-layer build-up under the guidance of projected patterns with the unpolymerized gel removed by washing. Similar to DMD, two-photon-based photopolymerization also uses photopolymerization of gels. But instead of manufacturing the 3D structure from bottom to top in a multi-layering manner, two-photon-based photopolymerization is capable of directly constructing complicated vascular models in only one step. Due to its great precision which can reach the scale of $20\,\mu m$ in vessel diameter, two-photon fabrication has been applied to the field of cell biology and bioengineering (46).

Biocompatible sacrificial material such as carbohydrate glass was printed to cast 3D vascular networks. Other than building the vessels directly, sacrificial networks can be embedded in the matrix along with tissue cells and dissolved rapidly, leaving perfusable tubes which can be later seeded with ECs (47). Kolesky *et al.* created thick human tissues (>1 cm) and perfused them with growth factors for long durations (> 6 weeks) to promote differentiation of hMSCs

toward an osteogenic lineage *in situ* (48). Nonetheless, ways to improve the accuracy of sacrificial material removal and build networks with more complex structures are waiting to be discovered. Besides, subtractive fabrication by laser ablation or 3D printing removal can also serve as a route to generate vessel channels. For example, sacrificial writing into functional tissue utilizes sacrificial ink which can later be evacuated to form perfusable vascular networks through tissue matrix, and the 3D biomanufactured vessel supported surrounding cardiac tissues for 7 days (49). Although there is no single solution that can completely solve the problem of organoid vascularization because of the presence of limitations in each strategy, including accuracy, dimension limit and construction cost, the variability of designs and unprecedented attempts provide ideas on future directions for researchers to explore.

Organotypic angiorganoid

Blood vessel organoid

Blood vessel organoids can be used to repair damaged or blocked blood vessels, form new blood vessels locally and restore tissue blood perfusion (43). Blood vessel organoids are amenable to model physiological structures, such as the blood–brain barrier (25), or pathological models, such as diabetic microangiopathy (44), and these models can be used for drug screening. In addition, some strategies of angiorganoid also need to construct the vascular system first and then directly introduce parenchymal cells into vascular organoids (45). Kusuma *et al.* induced iPSC to differentiate into early derivatives of the vascular lineage, which can mature into ECs and pericytes. This derived bicellular population can self-assemble to form vascular networks in an engineered matrix (50). The train of thought is to differentiate stem cells into ECs and vascular mural cells, and then, the mixed cell group can self-assemble to form organoids. Based on this line, Markou *et al.* generated spheroids consisting of EC/hiPSC-SMC from hiPSCs. The 3D spheroid co-cultures delivery *in vivo* rapidly generates a complex and functional vascular network (46).

Different from the above method, Wimmer *et al.* proposed a protocol for the generation of functional arteries, arterioles and venules from hiPSCs without the need to differentiate multiple cell populations (47). Since mesoderm progenitor cells could be the vascular progenitor cells, Colunga *et al.* described a human pluripotent stem cell-derived vascular progenitor (MesoT) cell of the mesothelium lineage, which can differentiate into the major vascular lineages and self-assemble into

vasculature when seeded onto decellularized vascular scaffolds (43). Organ-on-a-chip system is another common method of constructing blood vessel organoids, which can be used to form mechanical injury bleeding models (48), and distinguish vasculopathy and thrombosis among sickle-cell disease patients (49). Compared to the static culture system, microphysiological systems exert a shear force on the blood vessel organoid which might be more physiologically relevant. With the above-mentioned progress, however, the current blood vessel organoids could only mimic a handful of small blood vessels with two simple cell types (ECs and SMCs) let alone the big artery which is composed of three layers including intima, media and adventitia. In the future, more focus should be put on the recreation of large vessel organoids which are more clinically relevant.

Cardiac organoid

The heart is highly vascularized tissue due to the high consumption of nutrients and oxygen. Therefore, vascularization is essential for cardiac organoids. Noguchi *et al.* (51) created patch-like constructs fused by contractile cardiac spheroids, which contained cardiomyocytes, fibroblasts and ECs. And then, the cardiac patch was vascularized after transplantation into the heart of the rats.

Compared to scaffold-free techniques, scaffold-based techniques are more ideal for cell adhesion, distribution and responses. Lu *et al.* (52) engineered functional human cardiac tissue by repopulating decellularized mouse hearts with hiPSC-derived multipotential cardiovascular progenitor cells. However, because of the complexity of a decellularized heart, repopulating it efficiently and precisely with cells remains challenging.

Bioprinting is a promising technology to produce geometrically defined structures. Zhang *et al.* (53) constructed a 3D-bioprinted endothelialized microfibrous scaffold, on which the cardiomyocytes were seeded. But this bioprinted microfibrous structure was not perfusable. Redd *et al.* (54) created a cell organization with a vessel-like structure with a lumen of about 150 μm and firstly showed that bioprinted ECs can effectively develop vasculature in the transplanted tissues. Zhang *et al.* (55) created a scaffold with a built-in perfusable, branched, 3D microchannel network attached with ECs, and they engineered millimeter-thick cardiac tissues. It is notable that it can establish immediate blood perfusion after implantation due to the functional vascularized network yielded *in vitro*. Later, they modified the design of this bioscaffold to

enhance its capability, especially making it more favorable for cardiac tissue engineering (56).

While the tissue engineering technique approaches allow for high control of the end construct, they frequently do not faithfully represent the original cell composition and organization of the heart. Recently, self-assembling organoid technologies showed a promising potential to reproduce early cardiogenesis *in vitro*. Drakhlis *et al.* (57) reported foregut–heart organoids from hiPSCs with the vascular network. Hofbauer *et al.* (58) generated cardioids with a large internal chamber. Its separate EC lining, which has not been observed before, is crucial for the next stages of heart development. Lewis-Israeli *et al.* (24) created human heart organoids with vascular structures from hiPSCs, similar to the human fetal heart, through a three-step Wnt signaling modulation strategy and they also observed a point of high vascular branching that merits future investigation of heart vascularization.

Brain organoid

The brain is one of the most complex and important human organs. Many efforts were made to understand human brain development and disease. Compared with classic 2D neural cultures and animal model systems, brain organoids have more advanced cell composition, maturation and tissue architecture (59), consequently providing unprecedented opportunities for the study of brain 3D structures and diseases. Despite these advantages, brain organoids still have several limitations that need to be improved, for example, lack of vascularization which leads to the paucity of oxygen and nutrients that induce necrosis of the brain organoids (60).

Inspired by previous findings that central and peripheral neurons get consistent survival only when transplanted in a vessel-rich tissue (61), Mansour *et al.* first obtained *in vivo* vascularized human organoids model by transplanting human brain organoids into adult mouse brain (37). To extend the concept of vascularized brain organoid from *in vivo* to *in vitro*, Pham *et al.* re-embedded the brain organoid in Matrigel with ECs derived from the patient's own iPSCs. They found that the brain organoid was enriched by CD31-positive cells and capillaries had grown into the center of the organoid (33). Recently, Shi *et al.* obtained vascularized brain organoids by co-culturing hECs or hiPSCs with HUVECs, and it is worth noting that the vascularized brain organoid can form a connection with the host tissue when it is transplanted into a mice's brain (31).

One of the major limitations among those *in vitro* models is the segregation of blood vessels from the brain cells. The blood vessels tend to be confined in the periphery of the brain organoid without deep penetrations. Why is it? On one hand, the only addition of ECs themselves in the absence of perivascular cells might fail to form a robust blood vessel. By directed incorporation of MPCs into organoids, Wörsdörfer *et al.* generated vessels with mural cells, and typical blood vessel ultrastructure including endothelial cell-cell junctions, a basement membrane as well as luminal caveolae and microvesicles (34). On the other hand, lack of perfusion further set a brake on the blood vessels. To circumvent this, Cakir *et al.* developed a perfusion chamber to culture vascularized organoids, a technology to watch in the future (62).

Liver organoid

Several approaches have been explored to realize the vascularization of liver organoids. In 2013, Takebe *et al.* first generated vascularized LBs by co-culturing hiPSC-derived hepatic endoderm cells, HUVECs and bone mesenchymal stem cells (MSCs), which became vascularized and functional once transplanted into the mouse (29). Later, they developed a highly reproducible method to generate massive vascularized human LBs entirely from feeder-free hiPSCs using the microwell-array-based approach (63). However, they did not utilize hepatic stellate cells, which are critical for the basic regulation of liver homeostasis (64). In 2019, Li *et al.* established a reproducible self-assembly protocol to generate human LBs derived from hMSCs (64). Recently, Velazquez *et al.* applied integrated analysis and engineering of gene regulatory networks to generate hiPSC-derived multilineage human liver organoids, which contained a vast, interconnected network of the vasculature (65). Self-organized LBs in well arrays can be easily cultured, while they tend to have limited size and limited vascular networks.

By fusing hundreds of LB-like spheroids with a 3D bioprinter, Yusuke *et al.* reported a method for rapidly fabricating scalable liver-like tissue (66). Kang *et al.* fabricated a hepatic lobule structure, which is covered by ECs, using the preset extrusion bioprinting technique (67). Yang *et al.* generated 3D bioprinted hepatorganoids using HepaRG cells, which generated functional vascular systems *in vivo* after transplantation (68).

Bioprinting is capable of generating large-scale tissue constructs and modeling vascular networks within the tissues accurately, while the printing resolution is limited. Decellularized liver matrix can preserve the liver's general structure and vascular architecture. Baptista *et al.* successfully fabricated naturally derived 3D scaffolds with intact vascular networks based on decellularization, which human liver cells could repopulate using the vascular network (40). Modifying methods for liver decellularization, recellularization and differentiation of different liver cellular lineages of hiPSCs, Takeishi *et al.* generated functionally engineered human mini livers by repopulating the parenchyma, the vascular system and the bile duct network using five different cell types and performed transplantation in a rat model (41). Decellularization can generate whole-liver scaffolds of clinically relevant size. However, the shortage of organoids generated by decellularization such as immune responses after transplantation and long-term functional stability indicated a challenge in future exploration for clinical use.

Recently, Lai *et al.* reported a method to cultivate functionally vascularized hepatic tissues on organ-on-a-chip microdevices. Utilizing the 3D stamping technique with a synthetic polymeric elastomer, they engineered a microfluidic system with microchannels that can provide the stability of a perfusable vascular network (56). Although there is still a long way to go, these advances make it promising to further apply in regenerative medicine.

Kidney organoid

The kidney, as well as the cardiovascular system, comes from the mesoderm. As stated, it seems like the kidney is similar to the cardiovascular system. In 2013, Xia *et al.* (69) reported the differentiation of human pluripotent cells to ureteric bud progenitor cells. In 2014, Taguchi *et al.* (70) provided a deep insight into the developmental origin of the metanephric mesenchyme. Imitating the signaling pathways that occurred *in vivo*, they generated NPCs from iPSCs and formed 3D nephron structure *in vitro*.

As discussed in section 'Co-differentiation', vascular cells were emerging concomitantly with the development of kidney organoids. In order to fully establish the function of kidney organoids, transplantation is most widely used. After implantation under the renal capsule of mice, pre-existing networks in the kidney connect with graft-derived blood vessels. Compared with non-transplanted organoids, polarization and segmental specialization of tubular epithelium are observed (22). Additionally, Bantounas *et al.* have proven that after i.v. injection of fluorescent low-molecular-weight dextran, the signal was detected in tubules, demonstrating uptake from the glomerular filtrate (71). In the transplanted organoids, the blood vessels outside the glomerular structures have not

been extensively characterized. It is unclear to what degree their 3D structure and ECs resemble those *in vivo* (23).

The culture of kidney organoids under conditions of flow on a microfluidic device induces the enhanced differentiation and expansion of the endogenous EC population, resulting in the formation of vascular networks inside the organoids. These organoids have more mature podocyte and tubular compartments with enhanced cellular polarity and adult gene expression compared with that in static controls (4). A glomerular basement membrane was not demonstrated yet in the published transmission electron microscope images of organoid glomeruli, indicating that the interaction between the ECs and podocytes was not optimal.

Gut organoid

To generate HIOs, co-culturing endodermal cells and a mesoderm population increases the complexity, yet unable to resemble the native intestine. Holloway *et al.* found that ECs exist in HIO and declined over time, indicating that the standard culture conditions do not support robust ECs survival. They found a way to increase the proportion and longevity of the ECs within HIOs (27). Transplantation into the mouse mesentery enables HIO to grow larger than *in vitro* and to receive the vascular supply from mesenteric vessels, thus can be manipulated and mobilized with its vascular pedicle (72). In order to improve the conventional static culture, Rajasekar *et al.* designed a customized 384-well plate for the culture of up to 128 independently perfusable vascularized colon organoids *in vitro*.

Interestingly, they found an optimal gel formulation with 10% (v/v) matrigel in fibrin gel and an optimal medium with 50% EC growth medium and 50% colon media to support the long-term growth of vasculatures and colon organoids. Strikingly, the growth of organoids appeared to have accelerated after perfusion was established on day 5 (73). With the ability to grow vascularized colon organoids under intravascular perfusion, vascularized colon organoids could unveil new chances for modeling gut diseases or screening new therapeutics.

Islet organoid

Islet transplantation is an effective treatment for type 1 diabetes. To address donor shortages, islet organoids are being investigated as potential surrogates for human islets. However, given its poor vascular density, islet organoids in the subcutis will face poor engraftment, resulting in delayed glycemic control and poor survival.

Searching for new treatments to facilitate grafts' revascularization will be critical in transforming them into a viable therapeutic option.

Recognizing the need to improve graft survival in the s.c. space, many efforts have developed innovative approaches to establish a vascular connection with the host. Pancreatic islets isolated from adult mice and humans were capable of establishing vascular networks *in vitro* when co-cultured with HUVECs and hMSCs under appropriate conditions (30). The self-assembled organoid of human and mouse islets with ECs not only promoted functionalization (insulin secretion capacity) in culture but also massively improved post-transplant engraftment. A highly organized and resilient microvascular meshes were fabricated through a controllable anchored self-assembly method. The microvascular meshes promote the formation of functional blood vessels (74). Microvessels are small-diameter vessel fragments that can interconnect and inoculate with the host vasculature within days of transplantation (3). Employing readymade microvessels of adipose-derived may also result in the establishment of a fast and functional connection with the host vasculature and islet organoids and promote immediate normalization of glycemia (35).

Tumor organoid

Compared to vascular systems of normal tissues, the tumor vascular system is characterized by irregularly shaped discontinuous endothelium which is essential for tumor metastasis. Vascularized tumor organoids-on-a-chip open a window for recapitulating and observing the tumor intravasation when cancer cells gain access to the circulation (75).

Shirure *et al.* designed a microfluidic platform that mimics mass transport near the artery–capillary interface in the tumor niche. After the quiescent-perfused 3D microvascular network was created, patient-derived tumor organoids in an adjacent compartment were introduced. The tumor organoids could survive for several weeks and allow the dynamic and simultaneous observation of tumor progression including migration, proliferation, angiogenesis and intravasation (76). More recently, Silvestri *et al.* exploited the tumor organoid-on-a-chip and visualized the tumor–endothelial interactions. They visualized the integration of tumor into the endothelial lining to form a mosaic vessel which shed light on the mechanism of vascular recruitment and tumor intravasation (77). Although the *in vitro*-vascularized tumor-on-a-chip offers a cost-effective platform for

screening the drug candidates, the patient-derived organoid xenograft (PDOX) model could recapitulate the phenotypic landscape of a cancer-bearing patient. In that sense, the vascular components also constitute the immune suppressive microenvironment which could be a feasible target.

Summary and outlook

Blood vessels are not only an essential nutrient highway of different organs but are also responsible for providing the vascular cues to complete the specific organ functions. The transition from organoids to angiorganoids requires the collaborative output from developmental biology, stem cell biology, vascular biology and cutting-edge biofabrication technologies. In our mind, the lack of blood vessels from the organoids remains one of the biggest obstacles in the field. On one hand, the static culture lacks perfusion which could fail to energize the blood vessels to thoroughly infiltrate the organoids. On the other hand, co-differentiation concomitantly introduces the blood vessels during the organoid formation which ameliorates the segregation by mimicking natural development. With the goal of adding the temporally and spatially defined vascular network, inherent self-assembling combined with cell patterning, 3D printing and microfluidics could be applied to construct truly vascularized mini-organs while overcoming the segregation of blood vessels from the organoids *in vitro*. Compared to the organoid, angiorganoid offers another layer of sophistication and fidelity. Whether such perfusable angiorganoid represents a more reliable model for basic and translational applications warrants further research.

Author contribution statement

K W1, X J M and H F L researched data and wrote the manuscript. X W conceived and wrote the manuscript. K W provided funding support. S T Z, Z X H, W J K, Q H Y, R R W, Z H P, J Z and Y H C discussed the content and reviewed and/or edited the manuscript.

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