Lab on a Chip

CRITICAL REVIEW

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Vascularizing organoids-on-chip for perfused and personalized models

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Organoids represent one of the most advanced *in vitro* models for studying human physiology, development and disease. Their potential is very important and they have broad applications, but their impact is currently limited by persistent challenges such as incomplete maturation, batch variability, restricted long-range interactions and, critically, the absence of functional and perfusable vasculature. Integrating organoids into microfluidic platforms offers a way to overcome some of these constraints by providing a controlled and dynamic microenvironment with precisely tuned physical and biochemical cues. Among emerging strategies, vascularization stands out as a critical step toward improving organoid physiology and relevance: establishing stable, lumenized and perfusable networks within the 3D structure enables direct delivery of oxygen and nutrients, facilitates metabolic waste removal and promotes their maturation beyond embryonic stages. Achieving such models will require the combined expertise of stem cell biology, microfluidics, and biomaterials engineering to generate devices with organ-specific endothelial and stromal components, physiological flow profiles, and bidirectional anastomosis between endogenous and exogenous vascular compartments. This review discusses the biological rationale, current strategies, and technical considerations for vascularizing organoids-on-chip, highlighting their potential to improve physiological relevance, functional performance, personalization and translational applicability.

1. Introduction

Organoids are 3D self-assembled cell structures derived from embryonic stem cells (ESCs), adult stem cells (ASCs), or pluripotent stem cells (PSCs), that can represent a single organ or multiple organs, and are models enabling numerous readouts (morphological architecture, gene expression signatures, physiology) in comparison with 2D methods.[1,2] Through aggregation and self-organization, the aim is to recapitulate the structure and function of the *in vivo* counterparts, thereby providing physiologically relevant models for: studying organ development, disease modelling, and exploring transplantation strategies. Advances in the field have rapidly expanded the repertoire of available organoid types, driven by the need for more predictive *in vitro* systems. Their complexity can be tailored by: adjusting the number and type of constituent cell populations, the starting stage of maturation, while culture duration and conditions strongly influence maturation and functionality.[3] The global adoption of organoid technology has led to diverse methodologies—and equally diverse challenges. Indeed, as the technology continues to develop and spread, the scientific community is tasked with clearly defining these limitations and working together to identify viable solutions for patient applications.[4] Four major hurdles consistently arise in organoid research: incomplete maturation, insufficient or inadequate cell–cell and cell–matrix interactions, and the absence of vascularization.[2,3] The advent of microphysiological systems has enabled the development of dynamic culture, controlled flow dynamics, and continuous nutrient replenishment in small-volume environments.[5] Organoids-on-chip have gained increasing relevance due to their ability to precisely regulate physical and mechanical cues and to incorporate real-time monitoring with integrated sensors (*e.g.*, oxygen, temperature). Among the possibilities offered by microfluidic devices, a key focus is to establish a vascular network capable of supplying and draining the organoid. Indeed, hypoxic cores and necrosis develop because nutrient and oxygen delivery in organoids is diffusion-limited when diameters exceed ~200 µm.[6] Overcoming this diffusion-limit by introducing perfusable vascular networks has become a central objective in the field. Such active convection of nutrients and oxygen, in contrast to passive diffusion, holds the potential to markedly enhance organoid growth, viability, and functionality.[7]

In this review, we first outline the major limitations of current organoid models and then examine the techniques

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available to tackle the absence of a functional vascularization in organoids-on-chip systems, providing a critical viewpoint informed by recent work from our group.[8]

2. Organoids in static culture: a limited model

2.1. Maturity

Organoid formation recapitulates key aspects of embryonic development, meaning that cells are inherently immature at the outset and must acquire organ-specific functions as the organoid develops.[3] Two main approaches are used: either (i) co-differentiating all stem cells within a single 3D culture from the start, or (ii) pre-differentiating individual cell types in 2D monoculture to a defined developmental stage before assembling into an organoid.[4]

Maturity in this context encompasses transcriptional, structural, epigenetic, and functional hallmarks, many of which remain fetal-like *in vitro*. Most organoid models show incomplete maturation despite achieving structural organization and partial functional features of the target organ. Transcriptional analysis of kidney organoids, for instance, reveal profiles resembling first-trimester tissue, with expression of early developmental genes even though regional specificity of nephron structures was reached.[9] When considering the chromatin accessibility as a feature of epigenetic maturation of the kidney organoids, similar observation were made of foetal-stage maturation of cells, with differences even more pronounced in some nephron regions.[10] In intestinal organoids, maturation toward an adult-like state is markedly hindered *in vitro* compared with in-tissue development, even in models derived from adult stem cells.[11] Pancreatic organoids cultured *in vitro* exhibit altered metabolic activity (glucose response, respiratory activity) – a key functional readout for this model type.[12] For brain organoids, functional maturation depends on neuronal connectivity between distinct regions to establish physiological circuits, a process that remains challenging *in vitro*.[13]

Multiple studies have shown that transplantation into host animals can promote functional, morphological, and transcriptional maturation, as well as the emergence of additional cell types, highlighting the role of a physiological environment in advancing organoid development.[12,14,15] These findings underline the need to optimize *in vitro* culture system as conventional static methods often achieve high levels of complexity and function only after *in vivo* transplantation. Creating a supportive microenvironment will be essential to drive maturation without leaving the dish. In static culture, however, the surrounding matrix remains synthetic and reductionist, offering only a narrow subset of the biochemical and mechanical signals present *in vivo*. This dependence on artificial matrices represents a second major barrier, as these scaffolds only partially recapitulate native microenvironmental cues.

2.2. Microenvironment interaction

The importance of recreating an extracellular matrix (ECM)-like environment for organoid formation has long been recognized.[1] A common strategy is to embed organoids in a hydrogel containing matrix proteins, often supported by fibroblasts that secrete collagen, fibronectin, and laminin, rapidly assembling into a scaffold.[16] Many protocols still rely on Matrigel – a laminin- and collagen-rich hydrogel derived from mouse sarcoma – which remains one of the most widely used ECM-based matrices *in vitro*.[17] However, its tumour-derived origin introduces unwanted factors and significant lot-to-lot variability, potentially affecting experimental reproducibility.[18] Alternative approaches include supplementing natural or synthetic hydrogels with defined ECM proteins (*e.g.*, laminin, collagen, fibrin) or using scaffolds from decellularized tissues (dECM). dECM offers biochemical and mechanical properties closely matching native tissue and has been increasingly recognized for enhancing 3D organization, functional maturation, and phenotypic stability of organoids.[18,19] For instance, brain dECM, which closely mirrors the native tissue protein composition, has demonstrated greater batch-to-batch consistency than Matrigel,[20] while pancreatic dECM has been shown to enhance structural integrity compared with collagen matrices.[21] Nonetheless, dECM variability may still arise due to differences in tissue source[20] or heterogeneous ECM distribution within an organ, underscoring the potential need for standardized protocols.[22,23]

Beyond biochemical composition, the mechanical properties of hydrogels – stiffness, viscoelasticity, and tunability – also play a decisive role in organoid behaviour.[24,25] Mechanical parameters can be standardized through controlled fabrication protocols,[26] or quantitatively characterized by techniques such as atomic force microscopy, which probes the elastic modulus of the material at the microscale or rheometry, which provides bulk measurements of viscoelastic behaviour.[27] As matrix physical properties influence processes including stem cell fate decisions, cytoskeletal organization, and morphogenetic patterning, precise definition and reproducibility of hydrogel mechanical properties are critical for organoid culture.

In contrast to these structural cues, the biochemical composition of the ECM influences lineage commitment and functional behaviour through molecular signalling pathways. Both soluble factors (cytokines, growth factors) and insoluble ECM components, such as collagen and fibronectin assemblies, engage integrin receptors triggering signalling cascades that regulate gene expression and cell fate.[28,29] Incorporating additional support cells into the hydrogel can further enhance the microenvironment: fibroblasts contribute to ECM production and direct cell–cell contacts, while endothelial, immune, stromal, or cancer stem cells can be added depending on the research focus.[28,30–32] To reproduce more complex or spatially organized interactions, engineering approaches

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such as bioprinting now allow spatial arrangement of these supporting cells, for example controlled positioning endothelial cells (EC) around organoids to better replicate physiological interactions and functions.[21] While these strategies successfully capture local microenvironmental cues, they remain insufficient for modelling interactions that depend on long-range connectivity, such as circuit formation between distant brain regions. To overcome this limitation, assembloids – generated by fusing two or more region-specific organoids – provide a complementary approach that enable the study of developmental biology, inter-organ interactions, innervation, or vascularization by combining organoids from anatomically distant but functionally connected regions.[13,33–36] A recent study, for example, combined four hPSC-derived brain organoids – somatosensory, spinal, thalamic, and cortical neural organoids – to reconstruct the spinothalamic pathway, producing an interconnected system with synchronized electrical activity.[37]

Together, these engineering-driven advances – including defined hydrogels, dECM, bioprinting, mosaic organoids, and assembloids – are rapidly expanding the scope and physiological relevance of organoid models.[38]

2.3. Lack of vasculature

Nutrient renewal and oxygen distribution within the 3D structure also influence variability and growth. In static cultures, limited diffusion to the organoid core can induce central necrosis, and while continuous agitation can partially

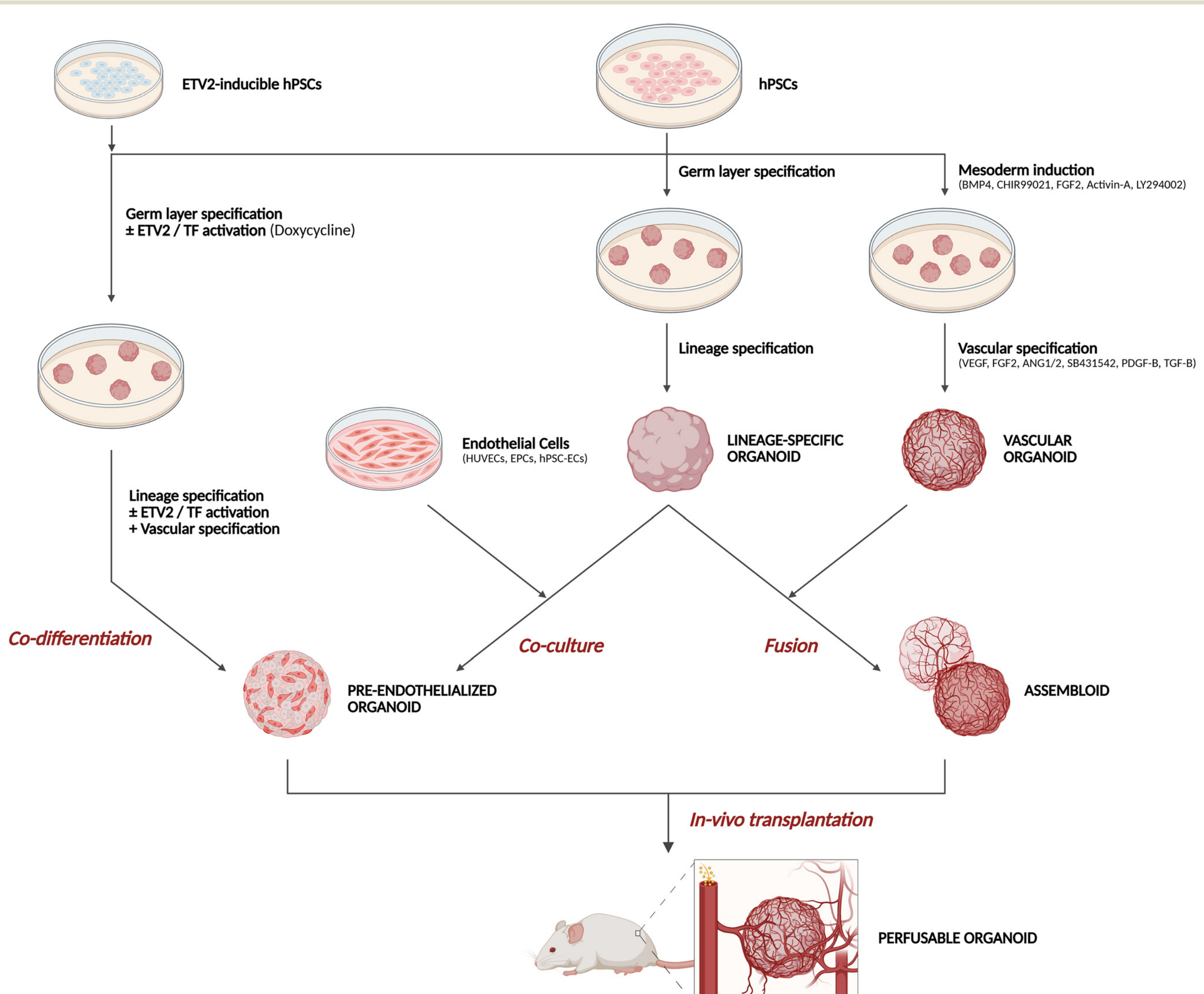

Fig. 1 Overview of current *in vitro* strategies to generate vascularized and perfusable organoids from human pluripotent stem cells (hPSCs). Lineage-specific organoids can be pre-endothelialized or vascularized *via*: (i) co-differentiation of parenchymal and endothelial lineages within the same hPSC aggregate, optionally driven by inducible ETV2 expression, (ii) co-culture with hPSC-derived or primary endothelial cells, (iii) fusion with independently generated vascular organoids. Upon transplantation into immunodeficient mice (*e.g.*, kidney capsule), vascularized organoids establish functional anastomoses with host vessels and become fully mature and perfusable. Created with Biorender.

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improve flow, it seems to remain insufficient to sustain long-term viability.[38]

Overview of current vascularization strategies in static culture. Vascularization offers an effective strategy to overcome these limitations, enhancing nutrient and oxygen delivery, reducing core cell death, and fostering physiologically relevant cell–cell interactions. Current main biotechnological approaches to organoid vascularization have been extensively reviewed recently, given the complexity of this challenge.[6,7] In static culture, the main strategies include: coculturing the organoid of interest with ECs, or combining it with a vascular organoid to form an assembloid, or inducing the simultaneous differentiation of organoid and vascular cells (Fig. 1). The choice of ECs source (human umbilical vein endothelial cells HUVECs, endothelial progenitors, or iPSCs-derived EC) and of supporting cells, including pericytes, smooth muscle cells, fibroblasts, or mesenchymal cells, critically influences the formation of a functional and integrated vascular network. Moreover, vascular cells display organ-specific transcriptional profiles, and even intra-organ capillary specializations; thus, using non-organ-specific ECs can result in a non-physiological microenvironment.[39,40] While endothelialisation with HUVECs can produce vascular-like networks, these do not always recapitulate functional vascular performance *in vitro*.

Representative studies. In brain organoid models, HUVEC incorporation increased tissue size, reduced hypoxia-inducible factor-1α (HIF1α) and cleaved caspase-3 expression, and thereby improved survival; however, unlike human brain microvascular endothelial cells (HBMECs), HUVECs failed to acquire brain-specific features such as blood–brain barrier marker expression (*e.g.*, *P*-glycoprotein).[41] As a result, they could not engage in the specialized interactions with astrocytes and pericytes already present within the organoid, and barrier-associated properties such as tight junction integrity and selective permeability were not restored, highlighting how endothelial identity critically shapes organ-specific vascular function. It is widely acknowledged that an endothelial network can be obtained by addition of HUVECs, but such networks are not necessarily stable nor perfusable, and often achieve functionality only after *in vivo* transplantation of the HUVEC-organoid system.[41,42] Quite recently, "reset" ECs generated by transient expression of the transcription factor ETV2 (ETS Variant 2), first developed by Lee *et al.*, represent a method that enables the generation of mature EC from postnatal fibroblasts.[43] This model of reset EC allows the formation of stable and lumenized vasculature, and has since been applied to multiple organoid systems.[44,45] When integrated with organoids, these reprogrammed cells can acquire tissue-specific features and support functional responsiveness.[44,46] An alternative approach – co-differentiating iPSCs into both organ-specific parenchymal and vascular lineages – remains challenging, as differentiation yields and endothelial maturation levels can vary considerably between experiments, introducing substantial variability.[6] Recently, two groups succeeded in creating vascularized cardiac, colon, intestine, hepatic and lung organoids, in which the induction of the vascular system was performed from the early mesodermal stage of the organoid, demonstrating how developmental biotechnologies could bridge a gap in organoid vascularization.[47–49]

The assembloid approach. Coculturing the organoid of interest with a vascular organoid – forming an assembloid – offers a promising route to physiological vascular integration. Blood vessel organoids (BVOs), originally developed to study diabetic vasculopathy, are generated by inducing mesodermal differentiation followed by vascular maturation.[50–52] These structures comprise pericytes, EC, mesenchymal stem-like cells, and hematopoietic cells, together forming a perfusable network capable of anastomosing with host vasculature upon transplantation. Advances in defining organ-specific vascular specializations, together with insights into the molecular regulators guiding BVO development, are paving the way toward the generation of BVO–organoid assembloids that more closely mimic the physiological interplay between tissues and their vascular supply.[39,53] To date, most studies of BVO assembloids have focused on brain organoids, where vascularization supports the high metabolic demands of neurons and contributes to modelling the complexity of the blood–brain barrier.[33,45] Similar strategies have been explored for tumoroids, bladder organoids, and pancreatic islets.[54–57]

Remaining challenges and unmet needs. Extensive bioengineering efforts continue to explore methods for generating pre-vascularized organoids, often with the aim of transplantation into animal models to assess functional integration.[45] However, these approaches have intrinsic limitations: vascularization alone rarely leads to substantial improvements in organoid maturation, does not fully restore a physiological cellular microenvironment (*e.g.*, contact-mediated signalling, mechanical cues), nor does prevent core necrosis under static culture. Even with established vascular networks, static conditions restrict nutrient and oxygen delivery throughout the full 3D structure. Collectively, these limitations underline a central point: achieving physiological tissue perfusion requires dynamic flow, which cannot be recreated through static vascularization strategies alone. This need has driven the increasing integration of organoids into microfluidic platforms, where controlled perfusion enables sustained nutrient delivery, waste removal, and the establishment of stable perfusable vascular networks.

3. A need for functional vascularization of organoids on-chip

Despite significant progress in organoids technology, achieving functional vascularization and sustained perfusion often remains reliant upon *in vivo* transplantation into animal models.[38,58,59] However, host–graft immune incompatibility, inter-species variability, ethical considerations and high costs limit the scalability and

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interpretability of such approaches.[6,60] Organs-on-chip systems offer a scalable, precisely controlled and ethically sound alternative, providing dynamic biochemical and biophysical cues that promote vascular integration and maturation *in vitro*.[61]

Organoids and organs-on-chip constitute two fundamentally different yet complementary strategies for modelling the complexity of human physiology and pathology *in vitro*. Organoids excel at recapitulating developmental self-organization and multilineage architectures, whereas organs-on-chip provide precise control over cell–cell and cell-microenvironment interactions, nutrient perfusion and external mechanical and electrical stimuli, offering functional advantages over static culture systems.[62,63] However, organ-on-chip models generally rely on combining pre-differentiated cells (often cell lines) to emulate native tissues through 3D matrix remodelling, and therefore frequently lack the cellular heterogeneity, phenotypic fidelity and physiologically relevant structural complexity characteristic of organoids.[61] Conversely, organoids remain limited in their

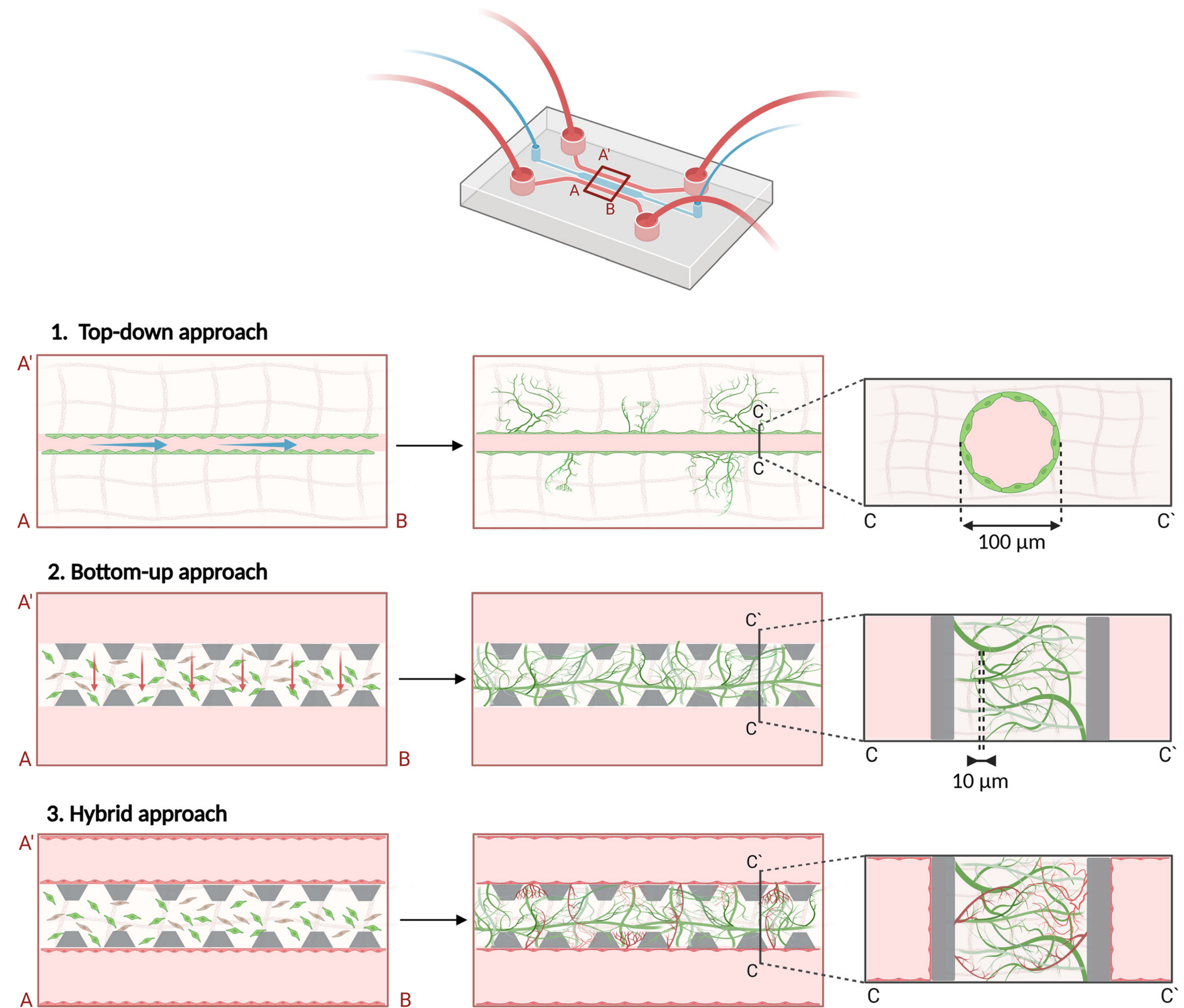

Fig. 2 Engineering strategies used to generate perfusable vessel-on-chip architectures. (1) Top-down, lumen-based models rely on pre-patterned conduits – obtained *via* microneedle withdrawal, sacrificial templating, viscous-finger patterning or 3D printing – that are subsequently endothelialized to form immediately perfusable (as indicated by the blue arrows) arteriole-scale channels (≈100 μm). Under pro-angiogenic cues, endothelial cells can sprout from these engineered lumens into the surrounding hydrogel. (2) Bottom-up, self-assembled microvascular networks embed endothelial and stromal cells within a permissive hydrogel, where interstitial flow between the lateral channels (as indicated by the red arrows) guides vasculogenesis-like migration, alignment, and lumen formation into capillary-scale meshes (≈10–20 μm). (3) Hybrid architectures combine endothelialized side channels with a central hydrogel chamber containing self-assembling microvasculature. Endothelial cells sprout from the patterned channels into the hydrogel following angiogenic principles – migration, matrix remodelling and tip-stalk cell dynamics – ultimately anastomosing with the self-assembled capillary bed to generate a continuous, multi-scale network. Together, these configurations span engineered arteriole-like conduits to biologically driven capillaries and form the basis of modern vessel-on-chip systems. Created with Biorender.

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ability to achieve functional vascularization and to integrate effectively within dynamic flow environments.

The combination of these two approaches offers a route to overcome the limitations inherent to each individually and to unlock a synergistic strategy for developing more faithful and functional tissue models for applications in regenerative and precision medicine. As suggested previously by Takebe *et al.*, balancing the complementary strengths of organoids and organs-on-chip – and identifying the optimal integration point – may provide a more effective strategy for generating high-fidelity, stem cell-derived *in vitro* organ models.[2] By combining organoid self-organization and cellular complexity with the dynamic microenvironment of microfluidic platforms, hybrid systems can more faithfully reproduce human tissue physiology while also providing a scalable alternative for complementing and potentially reducing animal studies for preclinical assessments.[64–66]

4. Vessels-on-chip

In order to obtain vascularized organoids on-chip, the development and understanding of techniques for endothelial and vascular systems on-chip is required. These systems are commonly referred to as vessels-on-chip, indicating vessel-like culture models implemented in microfluidics, with the ultimate goal of obtaining an *in vivo* relevant level of vascular organization and complexity.

4.1. Strategies for engineering vessels-on-chip

Two complementary engineering strategies have emerged to introduce a perfusable vasculature into organ-on-chip platforms: (i) a top-down, lumen-based approach in which pre-patterned conduits are endothelial-lined, and (ii) a bottom-up, self-assembly approach that lets endothelial and stromal cells build capillaries *de novo*,[67,68] as shown in Fig. 2.

The lumen patterning strategy begins by pre-defining micro-conduits whose geometry, branching and diameter are dictated by the fabrication technique. Hollow channels can be carved mechanically through microneedle withdrawal from soft biomaterials, moulded with sacrificial templates immersed in uncured hydrogel solution that are dissolved after gelation (*e.g.*, gelatine, alginate, Pluronic, carbohydrate glass), etched by viscous-finger patterning by pumping a less viscous fluid through an uncured hydrogel, or laid down additively by 3D printing and coaxial extrusion to build multi-plane, hierarchical bifurcating trees. Once the lumen is obtained, an ECs suspension is loaded to coat with a monolayer the inner walls of the conduit, generating an instantly perfusable "macro-vessel" whose shear profile and flow rate can be predicted from the channel dimensions and the fluid viscosity.[69–73] Technical advantages include precise and consistent control over vessel size and density, immediate perfusion, predictable intraluminal flow, and straightforward integration of sensors or pumps; practical constraints are (i) lumen diameters that rarely fall below 100 μm (small arteriole-scale vessels), (ii) non-circular cross-sections that disturb endothelial monolayers and create *in vitro* non-physiological artifacts, (iii) inconsistency in endothelializing small-sized or complex channels, (iv) limited capacity for adaptive remodelling (the vessel shape is fixed by fabrication) and (v) limited throughput due to technical challenges and manual labour.

Recent reviews catalogue rod-based luminal patterning as the archetypal "engineering-directed" strategy: geometry, branching and flow are fully determined by the artificially designed shapes and dimensions of the microchannels. This enables precise control over the morphology of the vasculature and predictable intraluminal shear stress, at the cost of a reduced biologic realism and limited micro-scale resolution.[67,68]

An alternative approach is biologically-driven and mimics the mechanisms of *in vivo* developmental vasculogenesis, the *de novo* formation of blood vessels from endothelial progenitor cells.[74] In the self-assembled microvascular networks strategy, ECs are co-embedded with stromal cells in what is initially a random spatial distribution inside a permissive hydrogel (collagen, fibrin, Matrigel, synthetic PEG/GelMA). Guided by intrinsic programmes and extrinsic cues – interstitial and intraluminal flow, vascular endothelial growth factor (VEGF) gradients, matrix stiffness – cells migrate, align and lumenize, generating microvessel networks with lumen diameters typically in the 15–50 μm range. Although larger than physiological capillaries (<10 μm), these structures are generally described as "capillary-like" meshes in microvascular-on-chip systems.[75–84] Within 3–7 days, the forming vessels can intertwine with one another and anastomose with adjacent medium channels, establishing perfusable microvascular beds that mirror key features of embryonic vasculogenesis. These networks exhibit continuous VE-cadherin junction belts, physiologically relevant permeability and dynamic responsiveness to inflammatory or metabolic stimuli. Together, these functional properties reflect the intrinsic strengths of the self-assembly approach – including robust self-organization, basement membrane deposition and ECM remodelling – while limitations include: (i) stochastic geometry and topology, (ii) reliance on mural-cell recruitment for long-term stability, (iii) gel compaction and (iv) less predictable shear stress distributions.

Together, these two strategies – and their hybrid combinations – provide a toolkit that spans macro-scale conduit precision to micro-scale capillary fidelity, enabling researchers to choose or blend methods according to the required vessel diameter range, flow control and fabrication resources. To bridge length scales, top-down arteriole-sized conduits are combined with bottom-up capillary beds in hybrid macro–micro architectures, in which self-assembled capillaries sprout in a hydrogel-filled chamber starting from endothelialized lateral medium channels.[85–90] The sprouting is governed by the biological process of angiogenesis, that refers to the formation of new blood vessels from existing ones. The process is orchestrated by the migration and

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proliferation of ECs promoted by angiogenic signals (*e.g.*, VEGF, Fibroblast Growth Factor FGF), degradation and remodelling of ECM by matrix metalloproteinases, formation and fusion of vascular sprouts, recruitment of support cells enhanced by angiopoietins (*e.g.*, Platelet-derived Growth Factor PDGF) and finally vascular maturation.[91] With the assistance of VEGF gradients, researchers can simulate the process of ECs degrading and invading the hydrogel chambers, yielding perfusable multi-scale networks encompassing artificially patterned macro-vessels anastomosed to self-assembled capillaries, better matching physiological hierarchy and flow distribution.[85,92] These hybrid macro–micro designs offer the most promising compromise to leverage the strengths of the two approaches, and should become the default rather than the exception as they likely represent the most versatile route for engineering perfusable vasculature on-chip. *In vivo*, vascular beds span a broad hierarchy – from large-calibre arteries to micro-capillaries – and exhibit organ-specific endothelial cell population and mural cell functionality. While current vessel-on-chip systems can only reproduce selected aspects of this diversity, hybrid architectures still enable the controlled formation of lumenized endothelial structures and early features of microvascular specialization, providing a functional basis for modelling key components of vascular physiology *in vitro*.

4.2. Vessels-on-chip limitations

Cell heterogeneity for fidelity, stability and lumenization. What is often referred to as a vascular network on-chip, frequently turns out to be purely an endothelial one. Indeed, the *in vivo* vascular network is highly heterogeneous in phenotype. A major limitation for the perfusion of current on-chip models is the lack of mural cells within the endothelial network, which fails to faithfully recapitulate the complex architecture of capillaries *in vivo*.[39] Notably, ECs embedded in a hydrogel alone typically form unstable vascular-like structures lacking well-defined lumens. The inclusion of stromal cells in co-culture significantly enhances microvessel formation and supports the development of stable lumens, as proved by Whisler *et al.* culturing ECs alone or with fibroblasts.[84] While direct physical contact is not required, stromal cells secrete a range of soluble factors—such as angiopoietin-1, angiogenin, hepatocyte growth factor, transforming growth factor-α, and tumour necrosis factor—that promote endothelial sprouting. However, lumen formation specifically depends on fibroblast-derived ECM proteins, including collagen I, PCOLCE, SPARC, IGFBP7, and βig-h3, which together increase matrix stiffness and provide the structural cues required for lumenogenesis.[93,94]

Building on the supportive role of fibroblasts, the addition of pericytes further enhances vascular maturation by promoting vessel stabilization, inhibiting regression, and refining lumen structure and function. Without mural support, vessels display elevated permeability, rapid regression and poor barrier protein expression, mirroring the *in vivo* observation that stable vasculature is always a composite of an inner endothelial tube and an outer mural layer.[95] Pericytes envelop capillaries and small venules; their PDGFR-β-mediated recruitment promotes basement-membrane deposition, tight-junction maturation and long-term patency, while their contractile phenotype fine-tunes microvascular diameter and flow resistance.[96,97] In the microfluidic setting, several studies co-culturing pericytes with ECs showed an overall enhancement in the barrier function.[98,99] Kim *et al.* demonstrated that inclusion of pericytes into their biomimetic vasculature increased numbers of junctions and branches yet greatly decreased vascular permeability as well as the vascular diameter as opposed to the EC monoculture.[99]

Smooth-muscle cells, instead, form concentric layers around larger arterioles and veins, providing the elastic strength and vasomotor control needed to withstand pulsatile pressure and actively regulate tissue perfusion, but they are not found in capillaries *in vivo*. In practice, coculturing ECs with pericytes, smooth-muscle cells or mesenchymal stromal cells is essential to produce differentiated, leak-resistant and functionally responsive vascular networks that approximate native physiology of the vessel of choice.[100–103]

Beyond cellular composition, it is also important to note that vascular phenotypes vary substantially across organs. ECs acquire organ-specific transcriptional, metabolic and barrier properties, and mural cell diversity further contributes to tissue-specific specializations such as blood–brain barrier function, sinusoidal permeability in the liver, or fenestrated capillaries in endocrine tissues.[39] Current vessel-on-chip models typically recapitulate early microvascular traits – such as lumen formation, barrier function and flow responsiveness – but only partially capture these organ-specific endothelial and perivascular identities. This incomplete specialization is partly attributable to the use of non-tissue-specific endothelial sources, such as HUVECs, which do not fully reproduce organ-dependent vascular phenotypes. Transitioning toward primary or hiPSC-derived endothelial and mural cells may therefore be necessary to achieve more faithful organ-specific microvasculature on-chip.

Integration of flow. Incorporating physiological flow in vessel-on-a-chip platforms is essential to faithfully mimic vascular structure and function *in vitro*.[104] Hemodynamic forces, especially wall shear stress (0.1–2 Pa), play a pivotal role in regulating EC behaviour, including gene expression, alignment, permeability, proliferation, and differentiation. Flow-mediated mechanical cues drive critical processes like vessel remodelling, lumen formation, and stabilization, which static conditions fail to replicate.[8] Studies have demonstrated that applying shear stress above 0.5 Pa is necessary to trigger cytoskeletal remodelling for angiogenic sprouting and cellular alignment, and to activate mechanosensitive signalling pathways.[6,104,105] Moreover, flow not only enhances vascular network maturation but also

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supports the emergence of arterial or venous identity depending on flow magnitude and pattern, with higher shear stress (>1 Pa) promoting arterial characteristics and lower levels (<0.5 Pa) favouring venous phenotypes.[105]

In addition to luminal shear stress, interstitial flow – the slow movement of fluid leaking through the ECM – plays a key role in providing directional cues that guide angiogenic sprouting and endothelial migration. Physiological interstitial flow rates (typically ranging between 0.1–10 μm s^{-1}), beyond aiding nutrient distribution and waste removal, exert low-magnitude mechanical forces that provide directional cues, promote ECM remodelling, and enhance capillary branching and elongation.[67,92,106,107] These effects arise from the activation of mechanosensory receptors such as PIEZO1, and complexes including PECAM, VE-cadherin, integrins, and VEGFR2. This activation triggers signal transduction pathways, such as the FAK pathway, which not only modulate cell phenotype but also influence the expression of key regulators of the surrounding microenvironment, such as the Matrix Metalloproteinase 1 (MMP1).[6,108–111]

Therefore, recreating physiological flow dynamics in microfluidic systems is essential for developing predictive and biomimetic vascularized organ-on-chip models. Flow can be introduced using various methods, including gravity-driven systems and motorized pumps. Among these, hydrostatic pressure differences or bidirectional rocking platforms are commonly used due to their simplicity, but they often generate irregular and direction-changing shear, which diverges from physiological *in vivo* waveforms. While under laminar flow ECs elongate and form tight junctions associated with reduced vascular permeability, turbulent flow can lead to weakened junctions and increased proinflammatory expression levels.[112] More advanced approaches, such as pressure-driven, syringe-driven or peristaltic pumps have been shown to provide a more developed and unidirectional flow, allowing finer control on the flow rate and shear stress applied.[113]

Functional assays. Functional evaluation of self-organized *in vitro* microvascular networks is essential to validate their physiological relevance and maturation status. Immunofluorescence is commonly employed to assess phenotypes and network connectivity, using markers such as CD31 and VE-cadherin, as well as tight junction proteins like ZO-1, and basement membrane components including laminin and collagen IV.[79,92,114–116] While these markers can highlight vessel maturity, functionality – particularly lumenization and perfusability – is best assessed *via* dynamic assays.

Perfusion of fluorescent dextrans (typically 3–150 kDa) or polystyrene microbeads through the vessel network enables live imaging of tracer transit, velocity fields, and bead trajectories, confirming the presence of continuous lumens and anastomosis with inlet channels.[79,85,89,116,117] These read-outs are widely accepted as gold-standard benchmarks for network perfusability; however, they lack standardization in tracer size or molecular weight, observation window duration, and data processing, limiting reproducibility and cross-study comparability.

Complementing these, diffusional permeability measurements using fluorescent tracers driven intraluminally provide a quantitative readout of barrier integrity, reflecting contributions from tight junctions, glycocalyx and mural cells interactions.[115,118–120] By tracking the accumulation of fluorescence in the surrounding hydrogel over time, the effective permeability coefficient (Pe) can be calculated, though it may be overestimated due to the neglect of convective transport. Notably, Pe values measured in 3D perfusable networks have been reported up to two orders of magnitude lower than those measured in transwell systems using the same cells, and to more closely match *in vivo* benchmarks.[4,79,83,116,121] This demonstrates that dynamic 3D conditions significantly enhance endothelial barrier performance, bringing it closer to physiological reality. Additionally, barrier transport of large macromolecules, such as albumin and therapeutic antibodies, has been assessed with paracellular permeability and transcytosis assays, providing insights into leakiness and selective transport properties.[121–124] For real-time, label-free monitoring of barrier tightness, trans-endothelial electrical resistance (TEER) could be measured using integrated electrodes flanking the vessel, offering a sensitive surrogate for endothelial integrity assessment.[125,126]

Ultimately, a combination of both structural and functional readouts provides a comprehensive and quantitative assessment of microvascular networks maturation and barrier function.

5. Vascularization of organoids-on-chip

5.1. Considerations and approaches

Current co-culture methods begin by seeding organoids on top of a pre-established vascular bed or by embedding them in the hydrogel precursor solution (Fig. 3). The latter may contain ECs and stromal cells to exploit a vasculogenesis-like self-assembly approach, or just the organoid, to obtain vascularization through angiogenic sprouting of ECs from a pre-lined, pre-patterned lumen (Fig. 3(1)).[127] Endothelial tip cells are either guided by VEGF gradients or chemokines to extrude through the matrix where the organoid is embedded in, extending towards it and potentially anastomosing with its endogenous vasculature.[4,39] Vascularization of organoids-on-chip and functional anastomosis could be potentially achieved by allowing the obtained perfusable vascular network to further sprout into the organoid. A proof of concept was demonstrated in numerous studies in which perfusable vascularization was achieved in spheroid models through vascular invasion.[128,129] Nashimoto *et al.* reported a vascularized cancer-on-a-chip model obtained by embedding a heterotypic spheroid comprising tumour cells, fibroblasts and ECs in a hydrogel solution seeded in the central chamber of a microfluidic chip.[128] Under the stimulation of

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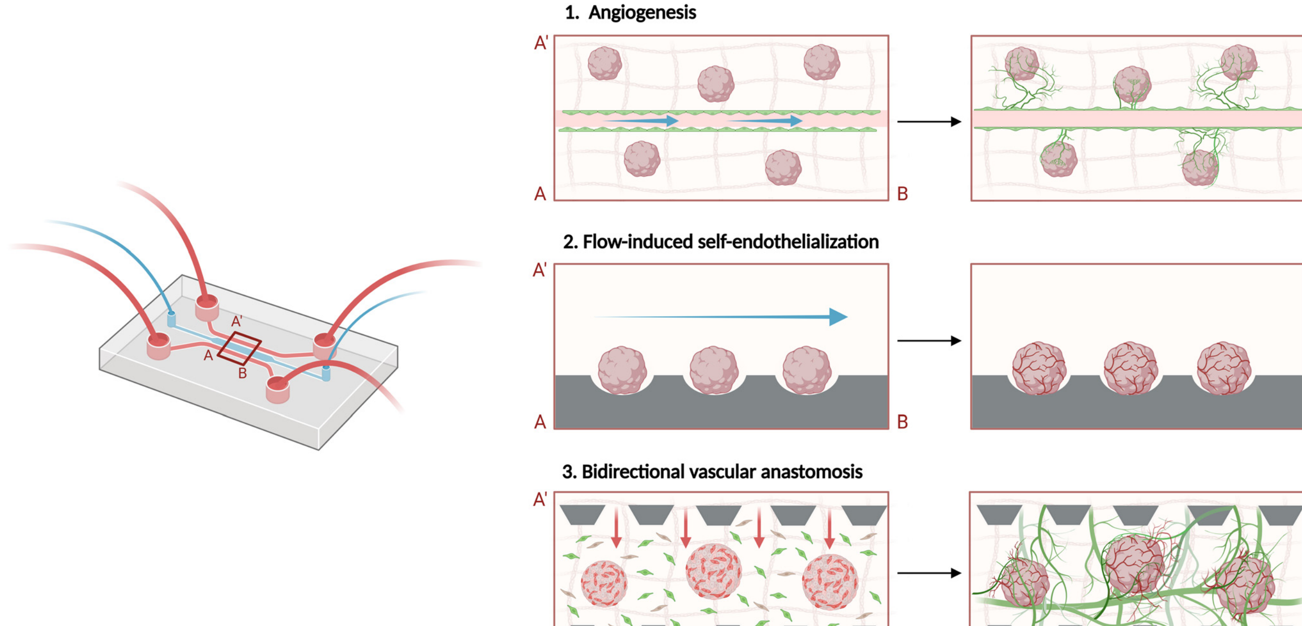

Fig. 3 Mechanisms enabling vascular integration of organoids on-chip. (1) Angiogenesis: organoids embedded in a hydrogel adjacent to an endothelialized channel can be vascularized through angiogenic sprouting. Guided by angiogenic cues such as VEGF gradients, endothelial tip cells sprout and migrate through the matrix toward the organoid and may invade and anastomose with it, as demonstrated in vascularized tumour spheroid models.[128] (2) Flow-induced self-endothelialization: controlled fluid shear stress applied on organoids can activate their endogenous endothelial precursors, stimulating the emergence of primitive intramural vessels that could grow outward once embedded into a permissive matrix.[130] (3) Bidirectional vascular anastomosis: when pre-vascularized organoids are co-cultured with self-assembled microvascular networks, endothelial cells can sprout from both the organoid and the surrounding vasculature, forming reciprocal connections that generate a continuous perfusable network. Although full intramural vascular integration remains rare in lineage-specific organoids, these mechanisms illustrate the strategies currently explored to achieve functional anastomosis in organoid-on-chip systems.[131] Created with Biorender.

angiogenic factors secreted by the tumour cells, ECs lining the lateral channels sprouted through the hydrogel growing toward the spheroid. After invading into the tumour spheroids, the vessels growing from both sides anastomosed, increasing the proliferative activity of tumour cells and reducing cell death in the spheroids. Remarkably, these functional anastomoses led to a fully perfusable network across the central gel, including the spheroid, as evidenced by successful perfusion of fluorescent dextran dye into the core of the spheroid.

Although achieving a fully perfusable vascular system connecting an external vascular network with an internal one has been demonstrated solely in vascularized tumour spheroids, recent research indicates that organoids derived from iPSCs can also secrete VEGF, suggesting potential for vascular integration beyond tumour models.[132,133] Examples of similar attempts at vascularization on-chip of parenchymal organoids have proved the vascular network to reach the vicinity of the organoid, sometimes enveloping it, but rarely extending deep into its core.[131,134–136] In a recent study, Shaji *et al.* employed a microfluidic chip to simulate neurovascular interactions, aiming at the vascularization of hiPSC-derived cerebral organoids. The study identified the VEGF-HIF1α-AKT signalling pathway through transcriptomic analysis as a key driver of angiogenesis within the system. While vascularization was initiated, it was inhibited after 10 days of co-culture, revealing critical insights into the limitations of *in vitro* vascularization.[131] Similarly, Schulla *et al.*[135] developed a co-culture model to address the vascularization of human small intestinal organoids (hSIO). By integrating organoids with ECs and fibroblasts in a fibrin/Matrigel matrix, the study demonstrated that co-culture significantly enhances organoid stemness and survival compared to monoculture, even though the vascular network did not seem to vascularize the organoid itself.[135] In a comparable setup, Salmon *et al.*[136] designed a chip with spatial separation between the endothelial and organoid compartments, requiring endothelial sprouting to reach hiPSC-derived brain organoids. While ECs successfully infiltrated the hydrogel chamber and perfusion was achieved, full vascular integration into the organoid remained limited.[136] Despite these major advancements, the vasculature in current organoids-on-chip models often exhibits primarily structural features and still faces challenges in achieving full functionality, such as sustained perfusion. This undermines the anticipated advantages of using vascularized organoid-on-a-chip as *in vitro* models for screening, given that the delivery of drugs or immune cells hinges on their transport through physiologically relevant vascular networks, mimicking the *in vivo* context. Nevertheless, recent developments in microfluidic design, co-culture protocols, and matrix engineering continue to push the field toward

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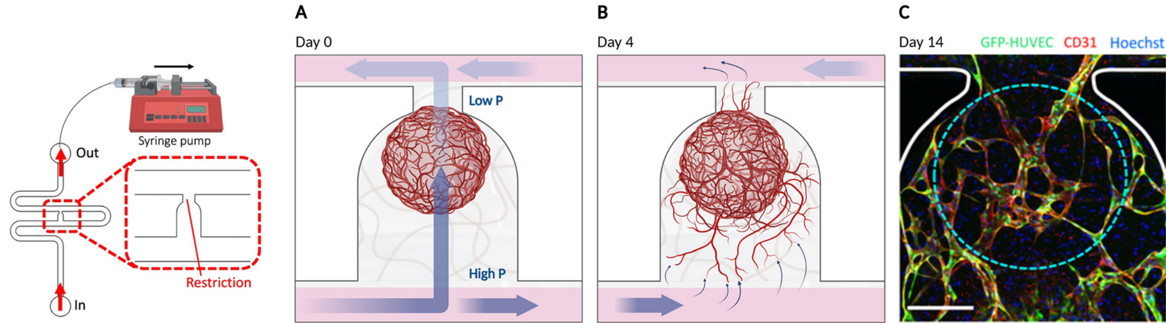
realizing this goal, and underscore the importance of addressing these challenges in future research.

In addition to supporting perfusion, the inclusion of endothelial components in on-chip models, even in the absence of a fully assembled vascular network, has also been shown to promote organoid maturation and functional refinement. Several recent studies demonstrated that their integration in co-culture systems enhances both functional and transcriptional maturation, underscoring the potential of vascular elements to drive developmental progression and improve organoid-based assays.[137–139] Landau *et al.* notably reported that co-culturing iPSC-derived cardiomyocytes with HUVECs, dental pulp stromal cells, and primitive macrophages enhanced both vascular stabilization and cardiomyocyte contractility.[137] In a gut model involving disaggregated intestinal organoids-on-chip, Ballerini *et al.* found that even without direct contact, the presence of EC influenced epithelial protein expression and improved tight junctions, suggesting a paracrine effect on barrier integrity.[138] In another study on hiPSC-derived kidney organoids on-chip, Homan *et al.* demonstrated that dynamic stimulation expanded their endogenous endothelial progenitor population and led to the formation of vascular sprouts within the organoid.[139] The emergence of these vascular structures supported epithelial polarity and ciliogenesis, with changes in protein expression toward mature tubule identity. Notably, the disruption of the endogenous VEGF gradient, either by VEGF addition or blockade, led to a loss of this mature phenotype, underscoring the critical role of epithelial–endothelial cross-talk. Interestingly, the interaction appears bidirectional: organoids can also enhance endothelial maturation. Co-culture systems have been shown to increase EC number, migratory capacity, and vascular markers expression. In a brain organoid model, endothelial and pericyte markers expression was upregulated compared to monoculture vascular systems,[136] while in bone organoids, the stage of organoid maturity influenced vascularization through the

Perfusion of BVO-on-chip

Sprouting of endogenous ECs under controlled flow stimulation



Common expected outcome: establishment of perfusable intraluminal vascular network

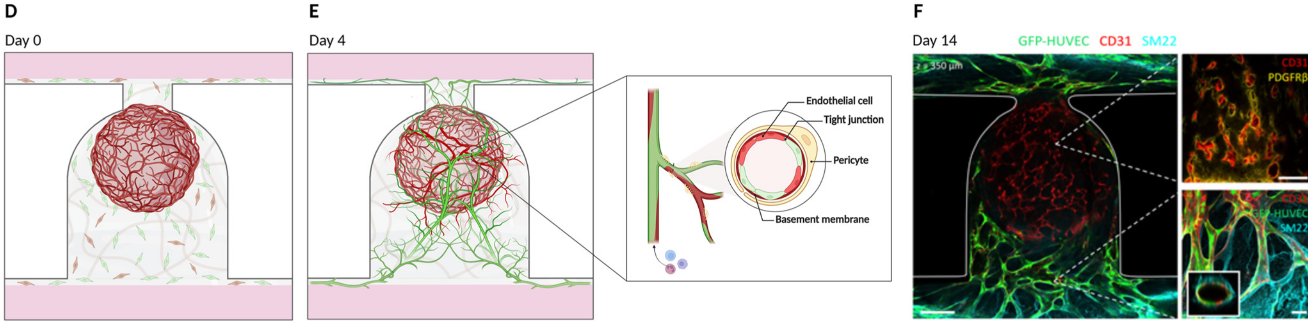


Fig. 4 Perfusion and vascular integration of blood vessel organoids (BVOs) on-chip. A schematic view of the microfluidic device with its U-shaped hydrodynamic trap used for passive organoid positioning, is included to contextualize the organoid position and perfusion setup used in this model. (A, B, D and E) Schematic representation of BVO (in red) on U-shapped serpentine chip and (C and F) confocal images from Quintard *et al.* illustrating two complementary modes of vascularization. (A) Under pressure-driven interstitial flow, endogenous endothelial cells (red) sprout from the BVO core and extend into the surrounding matrix; (B and C) once established, these nascent vessels are subjected to intraluminal shear stress, which promotes remodeling and functional maturation. (D) Schematic representation of BVO seeded on-chip together with HUVECs-GFP (green) and fibroblasts (brown) for the formation of an external network. (E and F) Anastomosis of BVOs with the external endothelial network enables the generation of continuous, lumenized vessels composed of endothelial cells ($CD31^+$, red) and perivascular support cells ($SM22^+$/$PDGFR\beta^+$, blue). Remarkably, the hierarchical structure of vascularized BVO-on-chip emulates the arteriole–capillary–venule transition observed *in vivo*, underscoring their physiological relevance and exemplifying the concept of hybrid macro–micro vasculature models-on-chip. Adapted from ref. 103, created with Biorender.

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secretion of pro- or anti- angiogenic factors.[130] Similarly, Bas-Cristóbal Menéndez *et al.* reported that the co-culture of kidney organoids with HUVECs enhanced endothelial migration and lumen formation, yielding a higher proportion of mature ($PECAM^+$) ECs compared to transwell conditions.[140]

These findings support the view that vascularization not only enables perfusion but also contributes, through both paracrine signalling and the flow-mediated biomechanical cues, to the biological relevance and maturation of organoids.

5.2. Challenges of organoids inclusion on-chip

Spatial localization. Early organoid-on-chip platforms typically relied on large, open hydrogel chambers that simply interface with flanking medium channels. Previous reviews of microvascular self-assembly underline that this big-chamber geometry is easy to fabricate but gives poor control over the final position of the organoids, which is stochastic, and generates highly variable fluxes, as flow must traverse the whole gel before reaching the construct. Haase & Kamm further note that perfusion across such centimetre-scale gel regions demands large medium reservoirs and offers little control over the shear landscape experienced by the forming vessels.[104] To regain positional control, hydrodynamic or capillary traps have been explored for passive localization of organoids-on-chip. For instance, Quintard *et al.* exploited a U-shaped recess off a serpentine channel to set the BVO position; because the trap's hydraulic resistance is lower than the main loop one when empty, the incoming organoid is automatically diverted and locked in place, achieving nearly 100% loading efficiency and allowing the channel walls to be uniformly endothelialized afterwards (Fig. 4).[8] The hydrodynamic trapping is dictated by a controlled flow rate of the injection, so this technique is not always applicable in case of PDMS-based chips, as the integration of a push syringe pump is not immediate. An alternative passive strategy for spatial localization of 3D structures on chip exploits micropillars arranged in circular arrays to form trapping sites in the central hydrogel chamber.[141–146] Rows of 50–200 μm posts pin the meniscus of liquid hydrogel so that a central gel lane forms while leaving adjacent perfusion channels open. By tuning pillar spacing and channel height, it is possible to confine gels robustly enough, although local depressions and defects at the pillar–gel interface remain common failure modes.[147,148] Compared with big-chamber layouts, U-shaped traps and micropillar confinements localize the organoid at a user-defined focal plane and permit reproducible shear conditioning, laying the foundation for high-content imaging and quantitative perfusion studies in vascularized organoids.

Extracellular matrix selection. The selection of hydrogel is a critical factor in the co-culture of organoids with capillary networks. Beyond serving as a structural scaffold for mechanical support, hydrogels provide essential biochemical cues including growth factors and adhesion molecules, that collectively guide organoid and vascular development. Natural hydrogels are the most widely employed materials due to their biocompatibility, superior cell affinity and accessibility.[149] However, it is important to note that organoids and vascular networks often require distinct culture microenvironments. While Matrigel, a basement membrane-rich matrix, is extensively used for organoid culture due to its support of epithelial organization and differentiation, it is generally inadequate for the formation of perfusable vascular networks, likely due to its limited remodelling capacity by EC.[150] In contrast, microvascular networks are commonly generated in collagen or fibrin matrices, where ECs and support cells can actively remodel the surrounding matrix, degrade the initial scaffold, and generate their own ECM, resulting in stable, lumenized vessels.[80,84,151–153] This disparity highlights the need to carefully optimize matrix compatibility in co-culture systems. In a study by Rajasekar *et al.*, a microfluidic platform was developed to co-culture patient-derived colon organoids with a self-assembled vascular network.[150] They observed that ECs successfully formed functional vasculature in fibrin but not in Matrigel, whereas colon organoids thrived in Matrigel but failed to grow in fibrin. To address this challenge, a composite hydrogel containing fibrin supplemented with 10% Matrigel enabled vascular sprouting and organoid development simultaneously. Despite the presence of perfusable vessels in the vicinity of the organoids, there was still no definitive evidence of functional anastomosis or intravascular perfusion within the organoid tissue itself. Composite hydrogel compositions should be investigated to find the optimal composition to suite vasculogenic assembly and organoid morphogenesis, while still accounting for the mechanical resistance needed to withstand the hydrodynamic stresses of the microfluidic setting (*e.g.* injection shear stress, meniscus pinning, long-term perfusion). Hybrid matrices comprising fibrin and ECM-like proteins (*e.g.* collagen, laminin, entactin, gelatine) should be considered for future applications: fibrin strain-stiffening fibres support rapid endothelial invasion and its proteolytic degradability allows dynamic remodelling, while retaining mechanical integrity under flow (*e.g.*, collagen alone needs a density higher than 6 mg ml^{-1} to resist flow on chip, which in turn hinders ECs migration).[131] Enrichment with basal-membrane like proteins offers native integrin ligands to guide endothelial alignment and organoid-supportive cues.

Medium modulation. In addition to the choice of the supporting matrix, the composition of the culture medium must also be carefully optimized for successful organoid–vascular co-culture. EC media typically contain essential growth factors such as VEGF, EGF, and FGF, which are critical for promoting endothelial proliferation, maturation, and the maintenance of functional vasculature. In contrast, organoid differentiation media are formulated with distinct sets of growth factors tailored to drive lineage-specific differentiation. A common strategy involves titrating various

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ratios of endothelial and organoid media to identify a formulation that supports the viability and development of both compartments, ensuring that no inhibitory interactions compromise organoid formation or vascular integrity.[4] Due to the difficulties encountered, in most co-culture systems, organoids are first generated separately using standard protocols, allowing pluripotent stem cells to differentiate and form self-organized structures. Introducing ECs too early could indeed interfere with organoid morphogenesis due to the influence of vascular growth factors and EC-derived paracrine cues.[7] Therefore, timing the initiation of co-culture is critical to preserve organoid identity while also promoting vascular integration.

6. Bidirectional functional anastomosis to achieve perfusion

6.1. Perfusable mesoderm-derived organoids

To obtain perfusion within the organoid, two complementary strategies should be combined: an inside-out mechanism should stimulate endogenous endothelial precursors to develop an internal vascular structure that sprouts outwards, while an outside-in mechanism driven by angiogenesis should promote the invasion of the organoid by the external exogenous vascular network.[4] Such mechanism closely mimics the process that occurs after *in vivo* transplantation of pre-vascularized organoids, where host vasculature invades the graft establishing a vascularized organoid through functional connections.[38,58,59] This mechanism is activated by pro-angiogenic factors, such as VEGF, secreted by the parenchymal cells within the organoid, as a natural response to the hypoxic conditions found in the core – a process that has also been observed in PSC-derived organoids.[60,61,76] These endogenous signals not only promote the ingrowth of external vessels but may also enhance the success of vascularization strategies such as co-culture with ECs (primary or iPSC-derived) or the co-differentiation of vascular lineages within the organoid itself.[6] The possibility to engineer the sprouting of endogenous ECs on-chip has been demonstrated by Homan *et al.*, who showed vascularization in hPSCs-derived kidney organoids under controlled wall shear stress (Fig. 3(2)).[139] Fluid stimulation and growth factors activated endogenous vascular development pathways in the organoid, stimulating the generation of ECs, thereby promoting the organoids` functional maturation and luminal structure formation. While they demonstrated that the vasculature within the organoid could embed and invade the surrounding ECM, these primordial vascular sprouts were not connected to an external vasculature for the establishment of a functional perfusable network. A follow-up study from the same group further advanced this concept by engineering a platform capable of supporting true intraluminal perfusion of kidney organoids *in vitro*.[154] In the dual-channel device, Kroll *et al.* embedded two parallel lumens: an upper one loaded with kidney organoids at day 10–11 of differentiation, and a lower one endothelialized with HUVECs to generate a bioengineered perfused macrovessel. Under controlled shear stress, endogenous ECs within the organoids expanded and migrated through the ECM toward the macrovessel. A key advance of this system was the demonstration of lumen-to-lumen anastomosis between organoid-derived microvessels and the artificial HUVEC-lined channel, enabling controlled perfusion of dextran and red blood cells through the organoid microvasculature. This model therefore represents the first *in vitro* demonstration of a fully perfusable kidney organoid, overcoming the major limitation of the earlier Homan *et al.* system, where endogenous endothelial sprouts lacked connections to an external vasculature and therefore could not sustain intraluminal flow.[139,154]

A conceptually related example of vascular integration in a mesoderm-derived system was reported by Arslan *et al.*, with a vascularized hiPSC-derived cardiac microtissue-on-chip (Fig. 3(3)).[134] In contrast to the kidney organoids described by Homan *et al.*, where ECs arise endogenously when stimulated by shear stress to expand and sprout, the cardiac model is generated through the engineered assembly of multiple hiPSC-derived lineages – cardiomyocytes, ECs and fibroblasts – thereby incorporating a pre-existing endothelial compartment. When these pre-vascularized microtissues were embedded within a vessel-on-chip platform containing an external network formed by hiPSC-derived ECs and pericytes, the two vascular compartments underwent bidirectional integration, with both outside-in invasion of the microtissue and inside-out extension of its internal vessels into the surrounding network, giving rise to lumenized hybrid vessels that supported particle perfusion under flow. Continuous perfusion further increased vessel density and the extent of anastomosis, and the presence of a connected vascular compartment modulated cardiac contractile dynamics and inflammatory responses *via* endothelial–cardiomyocyte crosstalk. Although these engineered microtissues do not fulfil organoid criteria of being assembled from pre-differentiated cells rather than generated through self-organizing developmental programs, they offer a compelling demonstration of coordinated anastomosis between a pre-vascularized hiPSC-derived 3D microtissue and an external, self-organized vascular network. A complementary approach to achieve directional functional anastomosis has been demonstrated using BVOs (Fig. 4D and F).[8] In the study by Quintard *et al.*, pre-formed hiPSC-derived BVOs connected to a self-assembled external vascular bed in a microfluidic device under hemodynamic flow, thereby permitting direct tracer perfusion through the organoid's internal capillary system, indicating that intraluminal perfusion could be established upon continuity between the two vascular compartments achieved.[8] Control conditions lacking the external microvascular bed did not exhibit intraluminal tracer flow, underscoring the requirement for a bidirectional connection to sustain perfusion. Extended perfusion culture for up to 30 days supported progressive vascular remodelling within the organoid, including increased

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network complexity, reduced hypoxia and ECM reorganization, features consistent with vascular maturation and stabilization. Transcriptomic analyses further indicated upregulation of endothelial identity and matrix-interaction pathways, while also showing downregulation of anti-angiogenic and immature endothelial markers, compared to non-perfused static controls. Although BVOs inherently contain vascular structures and therefore differ from lineage-specific organoids, this model shows that establishing physiological flow across a connected internal-external vascular interface can support the stabilization and functional maturation of organoid-derived microvessels. As such, this approach complements the mesoderm-derived models described above by providing a proof-of-principle for perfusion through a fully endothelialized organoid lumen, and also highlights the potential of combining vascular organoids with tissue-specific constructs as a route toward functional vascular integration.

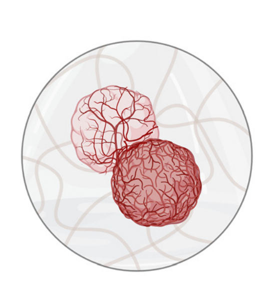
Taken together, these mesoderm-derived models illustrate that functional intraluminal perfusion can be achieved when organoid-intrinsic endothelial compartments – whether endogenously specified, engineered during tissue assembly, or provided through vascular organoids – are able to establish stable bidirectional anastomoses with an external vascular network. However, lineage-specific organoids derived from endodermal or ectodermal lineages generally lack such endothelial precursors, making these mechanisms more challenging to access.

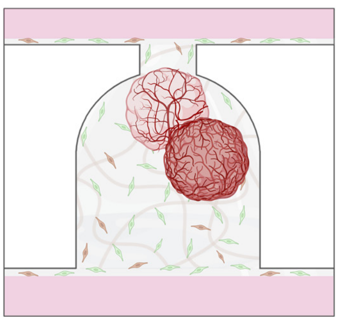
6.2. Assembloid-on-chip to vascularize lineage-specific organoids

While mesoderm-derived organoids can generate endothelial precursors that support the inside-out and outside-in mechanisms described above, most epithelial

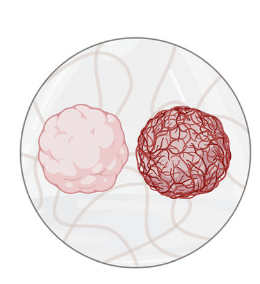
Towards a functional integrated model: assembloids-on-chip

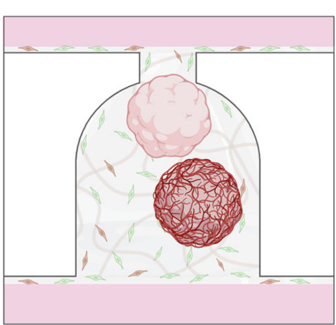
Strategy 1: pre-fusion off-chip





Strategy 2: direct co-culture on-chip





Common expected outcome: establishment of perfusable intraluminal vascular network

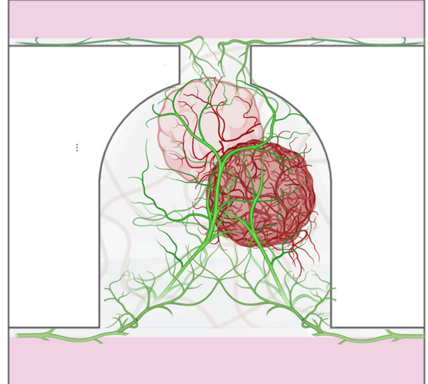


Fig. 5 Strategies for implementing assembloids-on-chip to achieve vascular integration. Two complementary routes can be envisioned to integrate parenchymal organoids with blood vessel organoids (BVOs) within microfluidic platforms. (Strategy 1: pre-fusion off-chip) the BVO and the target organoid are first fused in static culture, allowing vascular sprouts to establish internal connections with the parenchyma prior to loading into the chip. This approach ensures immediate vascular proximity and enables earlier onset of perfusion, reducing hypoxic stress during initial culture stages. (Strategy 2: direct co-culture on-chip) both the BVO and the parenchymal organoid are introduced into a shared hydrogel chamber, together with a suspension of endothelial (green) and stromal (brown) cells. Under continuous flow, angiogenic sprouts extend across the gel and progressively inosculate with the organoid. (Under, expected common outcome) despite differences in logistics, both strategies could converge on the establishment of a continuous, perfusable intraluminal vascular network that links the organoid vasculature with the external flow circuit, supporting maturation and functional assessment. Created with Biorender.

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and neuroectodermal organoids lack endogenous vascular lineages.

To address this limitation, several approaches have been explored *in vitro*. One strategy relies on co-culturing with primary or iPSC-derived EC, though integration often remains superficial and limited in depth.[4,58,155,156] Alternatively, the co-differentiation of ECs demonstrated in mesoderm-derived models has proven challenging in endodermal or ectodermal due to their distinct developmental trajectories.[48,157,158] Moreover, inducible genetic engineering strategies, with "reset" EC triggered by the expression of the transcription factor ETV2, can lead to the formation of vascular networks with patent lumens, yet the resulting networks typically lack the hierarchical architecture and stabilizing perivascular support required for sustained perfusion.[159–161] Finally, one promising approach involves the co-culture of lineage-specific organoids with BVO, which supply a self-organizing endothelial–pericyte compartment capable of sprouting toward and ultimately penetrating the target one.[57,162–165]

Building on these advances, we suggest that the employment of assembloids-on-chip could bridge the gap between vascular engineering in static biotechnology and the development of physiologically relevant, perfused tissue models-on-chip (Fig. 5). While lineage-specific organoids vascularization has been pursued through various *in vitro* strategies, their functional integration with dynamic flow environments remains limited. In contrast, Quintard *et al.* study[8] has demonstrated that BVOs are capable of establishing functional anastomosis with external endothelial networks under flow, enabling intravascular perfusion through the organoid. This precedent provides evidence that assembloids could be successfully integrated into microfluidic systems to achieve perfusion. Extending this concept, we propose that co-culturing the target organoid with a BVO within a microfluidic platform offers a promising modular route to overcome the intrinsic vascular limitations of epithelial organoids. Two complementary implementation strategies could be envisioned. In the pre-fusion (ex-chip) approach, the BVO and target organoid are allowed to integrate prior to seeding into the chip. This ensures that internal capillaries are already contiguous with the parenchyma at the time of loading, thereby simplifying spatial control and reducing the delay in establishing perfusion, which is advantageous for minimizing hypoxic injury during early culture stages. In contrast, the direct on-chip co-culture strategy involves introducing the parenchymal organoid, the BVO and a suspension of endothelial and stromal cells into a shared hydrogel channel. Under continuous perfusion, angiogenic sprouts from both vascular components can grow toward and inosculate with the organoid, guided by the biomechanical cues of the microfluidic environment. While both approaches aim to create a continuous intraluminal connection between the organoid and the external vascular network, they differ in practical logistics: pre-fusion provides immediate vascular proximity and earlier perfusion, whereas the on-chip strategy capitalizes on microenvironmental guidance and dynamic remodelling. Depending on the organoid type, required maturation stage and experimental constraints, assembloids-on-chip offer a flexible and adaptable framework to extend perfusion beyond mesoderm-derived models and to vascularize epithelial or neuroectodermal organoids that cannot autonomously generate vasculature.

7. Discussion

Despite rapid evolution of organoid technology, a major unmet need in achieving *in vivo*-like functionality has been the lack of mature structural organization, which is a direct consequence of absent functional vasculature. Without perfusable vessels, organoids rapidly develop necrotic cores, as metabolic demands outpace passive diffusion. Thus, incorporating functional vasculature for active convection of nutrients and oxygen seems indispensable for organoids growth and maturation beyond their embryonic stages. To date, perfusable vasculature within organoids has only been demonstrated through transplantation into host animals, where native vasculature either invades into the ectopic implant or anastomoses with pre-vascularized organoids.[58,59] Indeed, no *in vitro* approach has yet successfully demonstrated intravascular perfusion within lineage-specific organoids-on-chip, as most studies still rely on static diffusion or side-channel perfusion that fail to penetrate the core of the organoid. Achieving continuous and convective intraluminal flow with defined shear stress and stable barrier integrity therefore remains a major challenge, as is maintaining vascular maturity over time. Without physiological shear stimuli and cellular heterogeneity, *in vitro* vasculature tends to regress toward a foetal gene expression and leaky, immature phenotype. Additionally, the complex vascular architecture hinders control over continuous, directional flow both around and within the organoid.

In most vascularization strategies, HUVECs remain the EC type of choice due to their accessibility and compatibility with the foetal-like phenotype of hPSC-derived organoids. Yet, their primary origin, donor age, culture history (*e.g.* cell passage), and donor-to-donor variability limit their suitability for long-term modelling and tissue-specific maturation.[166,167] The next step toward greater physiological relevance and complexity should involve replacing primary vascular cells with iPSC-derived ECs, fibroblasts, pericytes, smooth muscle cells, and other relevant stromal components, tailored to both the organ-specific phenotype and patient genotype.[168] iPSC-derived ECs offer a renewable and customizable source of vascular and mural cells, and their use would enable the creation of fully autologous, vascularized organoid systems, advancing *in vitro* disease modelling and drug testing in the context of precision medicine.[169,170] Current personalization efforts are often limited to the epithelial compartment, as most parenchymal organoids are derived from patient-specific iPSCs.[171] However, for a truly integrated personalized

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model, the vascular, stromal, and immune compartments should also match the patient's genotype.

To meet these personalization goals while also addressing the limitations of avascular endodermal or ectodermal organoids, vascularized assembloids-on-chip composed of patient-derived components offer a promising solution. By integrating genetically matched endothelial and stromal components into perfusable assembloids, these models may achieve both vascular fidelity and immune compatibility. Importantly, such systems extend beyond modelling vascular pathologies and are well suited to investigate disease processes in which microvascular dysfunction plays a mechanistic role, including diabetic microangiopathy (for which BVOs were originally developed), neurovascular dysfunction and blood–brain barrier impairment, tumour vascularization and metastatic extravasation.[51,172–174] They could also provide a generalizable strategy to support the maturation, metabolic stability and functional performance of a wide range of lineage-specific organoids – including neural, hepatic, renal, pancreatic, intestinal and cardiac models – where sustained perfusion, controlled microenvironmental cues and vascular-parenchymal cross-talk are essential. This also opens the door to studying how organ-specific vasculature shapes tissue development, barrier properties, differentiation trajectories and drug responsiveness in a fully human context. When implemented on-chip, such platforms not only enable sustained perfusion, but also open the door to functional studies that require a dynamic vascular interface like immune cell trafficking, leukocyte adhesion and extravasation, endothelial activation under defined shear stress, and directional inter-organ communication mediated by physiologically relevant flow. They would also support a wide range of quantitative assessment of both vascular and tissue-level readouts: barrier integrity and selective permeability, angiogenic and matrix-remodelling dynamics, inflammatory and cytokine signalling, flow-dependent metabolic exchanges, endothelial mechano-transduction, and shear-induced modulation of organoid maturation and function; parameters that are difficult to access *in vivo* and highly relevant for disease modelling and therapeutic testing. In addition, such systems enable controlled delivery of therapeutics, nanoparticles or cytokines, allowing assessment of drug penetration, vascular transport, accumulation and toxicity in a fully human context, capabilities that are highly relevant for preclinical screening and mechanistic disease modelling. Looking ahead, moving toward interconnected and interdependent systems, multi-organoid-on-chip platforms linked by perfusable vasculature could more faithfully replicate human systemic physiology. Indeed, organoids naturally and intrinsically recapitulate much of the cellular diversity and intercellular cross-talk found in native organs, yet modelling higher-order dynamic interactions remains a significant limitation of current organoid systems. Currently, most multi-organoids-on-chip models rely on microfluidic arrays of isolated compartments connected by recirculating media rather than by functional vascular bridges.[175–179] This constrains inter-organ communication to paracrine signalling (*e.g.*, cytokine-mediated cross-talk), and fails to replicate the directional distribution of soluble factors, immune cells, and therapeutics *via* physiologically meaningful perfusion routes. Introducing vascular interconnections between organoids would enable circulation-like flow, essential for modelling ADME processes and systemic immune responses across multiple tissues.[180] Functional vasculature would thus serve not only as a conduit for perfusion, but also as a dynamic bridge for drug delivery, immune cell trafficking, and inter-organ signalling, ultimately improving the accuracy of toxicity and efficacy assessments of personalized therapy approaches. Within the framework of the 3Rs, these platforms currently contribute primarily to reduction and refinement, by supporting mechanistic studies on human-relevant pathology and decreasing reliance on animal models, and may progressively enable partial replacement in disease areas where human-specific responses cannot be adequately recapitulated *in vivo*. Notably, the translational relevance of human vascularized organoid systems is beginning to be recognized at the regulatory level. Recent reports indicate that efficacy data generated using human vascularized organoid models have contributed to the first FDA Investigational New Drug (IND) acceptance supported by an *in vitro* vascularized organoid platform, underscoring their potential to complement or, in specific contexts, partially replace animal studies during early-stage therapeutic development.[181]

Realizing this vision will require compatible media formulations, optimized co-culture conditions, and strategies to mitigate metabolic waste accumulation between connected compartments, advancing the translational readiness of vascularized organoids-on-chip for future patient-specific clinical applications.[182]

Conclusions

Vascularised organoid-on-chip systems have passed the stage of conceptual novelty, as progress over the past years has lifted vascularized organoid technology from proof-of-concept to a promising micro-physiological test-bed, yet the field still confronts fundamental hurdles that limit fidelity and translational value. There is now a clear need for rigorous standardisation, organ-specific and patient-matched cell sources, and mechanically active microenvironments to fulfil their promise as predictive human surrogates. The next leap will come not from a single fabrication technique, but from the coordinated integration these elements into cohesive, user-friendly platforms that can exit the engineering lab and enter routine biological, pharmacological and regulatory workflows.

Author contributions

BM wrote the original manuscript, and prepared the graphics. PDG wrote the introduction and provided critical

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input throughout the manuscript. BM, PDG, XG and ET conceptualized, edited and revised it. The work was fulfilled under XG and ET supervision.

Conflicts of interest

There are no conflicts to declare.

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

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