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Review Article

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Vascularized Organoid-on-Chip Platforms: Expanding the Bioengineering Frontiers with Microfluidics

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Abstract
Organoids-on-a-chip (OoC) technology is a pivotal advancement in biomedical research, engineered to meticulously replicate the intricate physiological functions of organs within controlled in vitro microenvironments. A critical component for elevating the biological fidelity and physiological relevance of these advanced platforms is the integration of a functional vascular system, vital for delivering nutrients and oxygen, waste removal, and intricate cell-cell communication. In this context, microfluidic technology has proven to be a potent tool. Its unparalleled precision in controlling fluid dynamics and engineering microscale architectures facilitates the creation of perfusable and functional 3D microvasculature within a wide array of OoC systems. The article begins with a comprehensive overview of approaches to harness microfluidics for such platforms, and is structured to highlight the key advantages and challenges associated with the technology. Furthermore, the nuances and progress in the realm are reflected through a discussion on recent exemplary studies, illustrating the diverse biomedical applications of microfluidics-driven vascularized OoC (vOoC) systems.

Keywords:
organoid-on-a-chip; vascularization; microfluidics; disease modeling; 3D microvasculature; perfusability; tissue engineering

1. Introduction

Recent explorations into smart biomaterials with robust biophysicochemical attributes, including unique nanoscale features, have significantly advanced the field of bioengineering [1,2]. Concurrently, progress in various rapid prototyping approaches [3] and ongoing research into the biofabrication of physiologically relevant biomimetic structures using 3D bioprinting, electrospinning [4], and microfluidics [5–7] has significantly propelled this field forward. Organoids, as innovative 3D self-organizing cell cultures from various lineages, have gained significant attention for modeling diverse tissues and organs [8]. They significantly advance understanding of developmental and disease biology, offer patient-specific insights into various health threats, and hold promising potential for regenerative cell therapies. However, the absence of a functional vascular network can be a significant limitation in fully realizing their potential to replicate in vivo physiology and support clinical translation. Vascularization is indispensable for delivering essential nutrients and oxygen as well as the recruitment of immune cells within engineered tissues, thereby regulating tissue survival, regeneration, and homeostasis [9]. An inadequate blood supply can lead to tissue necrosis. Similarly, vascularization facilitates the removal of metabolic waste products.

The human circulatory system is a complex network of blood vessels, ranging from large arteries and veins to tiny capillaries, each with vital roles in various bodily functions. Blood vessel formation primarily involves vasculogenesis, the formation of new blood vessels from progenitor cells, and angiogenesis, the growth of new vessels from existing ones. Angiogenesis involves the coordinated activity of specialized tip cells that lead the sprouting process and stalk cells that follow [9]. This

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intricate process is essential for various biological functions, including growth and development. Furthermore, it is well established that neovascularization profoundly affects wound healing, impacting the quality of new tissue formation [10]. While biomaterial-based approaches can be employed to enhance angiogenesis or vasculogenesis in a spatially, temporally, or spatiotemporally controlled manner, cell-based strategies enable the targeting of neovascularization by modulating the responses of angiogenic cells at the wound site. A key concept in vascular network formation is the establishment of a hierarchical organization, where vessels of different sizes and functions (arterioles, capillaries, and venules) are spatially and temporally coordinated. This hierarchy is essential for efficient blood flow and tissue perfusion. Endothelial cell (EC) heterogeneity, differential expression of angiogenic factors, and dynamic cell–cell interactions contribute to vessel hierarchy formation [11]. Additionally, mechanical forces like blood flow patterns and tissue stiffness influence the organization and remodeling of vessel networks.

The creation of vascularized organoids employs several advanced bioengineering strategies [12]. A widely used approach involves (a) co-culturing ECs with supportive cells like pericytes and fibroblasts, which stimulates the self-organization of vessel-like structures within the organoid. Alternatively, (b) integrating lineage-specific organoids with pre-formed vascular organoids enhances vascular incorporation and functional integration. The technique of (c) organoid co-differentiation guides stem cells to simultaneously develop into both organ-specific and vascular cell types, thereby promoting synchronized tissue development. (d) Embedding organoids into microfluidic devices (organoid-on-a-chip, OoC) allows dynamic perfusion and improved physiological fidelity [13], while (e) 3D bioprinting enables the precise spatial placement of cells and materials to fabricate organoids with predefined vascular networks. Each method offers distinct advantages: co-culture and co-differentiation are biologically intuitive and mimic natural developmental processes, vascular organoid integration accelerates network formation, OoC provides controlled perfusion and real-time analysis, and bioprinting offers architectural precision. However, limitations persist—self-assembly lacks control over vessel geometry, vascular organoid fusion may face integration challenges, co-differentiation requires finely tuned protocols, organ-on-chip systems demand technical expertise and may face material constraints (e.g., absorption of small molecules by polydimethylsiloxane (PDMS) [14]), and bioprinting may affect cell viability and depends on evolving bioinks. Together, these methods reflect a balance between biological realism and engineering control, with each suited to specific applications in disease modeling, tissue engineering, or drug testing.

Particularly, vascularized OoC (vOoC) models have opened new research avenues, enabling the study of drug pharmacokinetics and pharmacodynamics as well as the investigation of vascular-related diseases, all within a more physiologically relevant in vitro environment [9,15]. These are complemented by advancements in detection, analysis, and organoid imaging, particularly with artificial intelligence (AI), thereby enhancing OoC functionality and drug screening [7]. Although the number of publications and patents on OoC technology is constantly increasing, challenges remain, including multi-organ integration and whole-body modeling (an endeavor towards a human-on-a-chip model). Nevertheless, the vOoC platforms enable researchers to observe dynamic vascular processes, such as angiogenesis and cell interactions, in real time. Similarly, vessel-on-a-chip/ vasculature-on-a-chip (VoC) models may be harnessed to study oxygen and nutrient transport as well as mimic various functional tissue/vascular barriers (e.g., blood brain barrier, alveolar-capillary interface, renal vascular tubular unit, human placenta) [16]. Against this backdrop, the following section focuses on microfluidics-assisted vOoC platforms.

2. Advantages and Approaches of Microfluidics Assisted vOoC Platforms

2.1. vOoCs—The Advantages

A comparative assessment of different contemporary biological research models by Chaudhari et al. (2025) [17] revealed distinct pros and cons. While animal models provide dynamic, complex cell-cell interactions and intrinsic vasculature, they introduce ethical considerations and lack precise control or high-throughput screening. Traditional 2D cultures are severely limited across various parameters. Organoids improve ethical considerations and screening but typically lack intrinsic vasculature, controlled microenvironments, and dynamic mechanical cues. In contrast, microfluidic 2D cultures and especially OoC models present significant advantages. As mentioned previously, these advanced platforms facilitate in vitro vascularization, offer precise microenvironmental control, ensure continuous nutrient supply, and enable the emulation of mechanical cues and real-time data sensing, greatly enhancing physiological relevance and research throughput. Thus, microfluidic chips have become a highly effective platform for in vitro microvasculature modeling, owing to their numerous advantageous features [18,19]:

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(a) Microfluidic systems offer high spatial and temporal regulation of the cellular microenvironment, enabling fine manipulation of cell behavior and tissue development.
(b) They enable the formation of perfusable microvessels ranging from 20–50 μm in diameter by modulating interstitial flow within the extracellular matrix (ECM), thereby closely mimicking the dimensions and functional characteristics of native human microvasculature.
(c) Through emulation of mechanical cues and creation of controlled gradients of biochemical cues such as growth factors and signaling molecules, which are essential for cell differentiation and tissue patterning.
(d) They allow real-time data sensing, precise control of flow rates and shear stresses, facilitating physiologically relevant fluid flow patterns across the engineered tissue.
(e) Continuous perfusion ensures a constant supply of oxygen and nutrients while efficiently removing metabolic byproducts, thereby promoting sustained cell health and function.
(f) Microfluidic models can replicate the hierarchical architecture of vascular networks, offering a physiologically relevant model of vascular complexity. They can create 3D vascular architecture, including the circular cross-section of blood vessels and luminal/apical-to-abluminal/basal polarity, both of which are crucial for the directed secretion of proteins involved in lumenogenesis.
(g) These systems can be tailored to study a wide range of physiological and pathological aspects of vascular biology, making them valuable tools for translational research and drug testing.
(h) Techniques, such as injection molding and laser cutting, can facilitate the mass production of these chips (with relatively simple architecture), making them suitable for high-throughput screening of vascularized organoids.

2.2. Strategies Towards Developing vOoCs

Several microfluidic techniques for engineering vOoCs exist [17]. Droplet microfluidics enables high-throughput assays by isolating organoids in controlled microenvironments, while perfusion microfluidics overcomes diffusion limits by mimicking blood flow. Integration with hanging-drop systems supports 3D organoid formation and vascularization. Advanced co-culture platforms promote angiogenesis and tissue viability. Furthermore, automated microfluidic systems facilitate large-scale organoid production with integrated real-time monitoring, providing scalable and precise platforms for applications in regenerative medicine and personalized therapeutic development. A detailed discussion of these is beyond the scope of this article.

Nevertheless, the microfluidics approach for vascularization can be broadly categorized into the wall-trapping method and the microencapsulation method [15], while biomimetic vasculature is also being created using innovative techniques, including viscous fingering and the application of leaf venation patterns [13]. Varma and Fathi's categorization of various in vitro vascularization strategies in OoC platforms [13] illustrates the inherent trade-off between simplicity/researcher control and physiological fidelity.

The most straightforward method, EC-lined channels with fluid flow connected to a culture chamber, offers ease of implementation and precise control over perfusion, making it suitable for basic flow studies or medium exchange. Based on the material used, the fabrication approach for microchannels can be classified as [18]:
(a) Hydrogel-based: Microneedle-based method (creates single channels); Micropatterned, planar hydrogel method (creates single-layer channel network); Dissolvable material-based sacrificial micro-molding method (creates multi-layer channel network).
(b) PDMS-based: Requires coating with extracellular matrix (ECM) proteins like laminin, fibronectin, and collagen IV to enhance cell adhesion.

However, a fundamental limitation of this approach lies in its inability to replicate the complex, hierarchical branching characteristic of native vasculature, thereby limiting its applicability in comprehensive studies of vascular development and pathology. Conversely, methods relying on EC-based self-assembly to form microvasculature under static conditions represent a significant step towards biological realism by mimicking in vivo vessel formation. While this offers a greater faithful morphological representation of microvasculature, the critical absence of fluid flow effects is a major physiological shortcoming. Interstitial fluid flow and hemodynamic forces, such as shear stress, are crucial regulators of EC behavior, orientation, morphogenesis, and vessel maturation in vivo. Therefore, static self-assembly models may fall short in addressing questions where mechanotransduction or flow-mediated remodeling is paramount.

The artificial biomimetic network creation attempts to bridge this gap by allowing researchers to design and control complex network geometries, potentially offering a more similar in vivo-like structure than simple channels. However, since the network is not formed de novo through biological self-organization, there lies a question about the extent to which its functional and molecular characteristics truly mirror those of native vasculature. Furthermore, the potential for interactions between cells and synthetic scaffold materials, if not sacrificial, may introduce an addi-

tional layer of complexity and potential confounding factors.

Ultimately, self-assembled microvasculature under flow conditions emerges as the most physiologically relevant approach. By combining the natural process of biological self-assembly with the essential physiological cue of fluid flow, this method holds the greatest promise for faithfully recapitulating in vivo vascular networks. Such high fidelity is crucial for developing predictive models of vascular diseases, assessing drug efficacy, or understanding complex angiogenesis.

A critical component of vOoC systems is the incorporation of hydrogel-based channels, which serve both as a scaffold for vessel formation and as a conduit for nutrient and waste exchange. This structural and functional integration is essential for sustaining tissue viability and modeling vascular phenomena under controlled experimental conditions [19]. However, the significant technical challenges associated with its successful implementation, particularly in achieving stable and controlled perfusion across multiple interconnected compartments in multi-organ chips, currently limit its widespread adoption. The choice of vascularization method must, therefore, be strategically aligned with the specific research question, balancing the need for biological complexity with practical feasibility and throughput.

3. Exemplary Endeavors and Applications

Microfluidics technology has been employed for various biomedical studies, e.g., a heart-on-a-chip platform for in vitro cardiomyocyte monitoring (crucial for cardiac drug screening and disease modeling applications) [20] and sensors-integrated gut-on-a-chip model, simulating the intestinal barrier for in situ monitoring of Hg (II) transport and absorption [21] merit mention. Specifically, microfluidics-assisted vOoC platforms have been harnessed to provide a comprehensive insight into the angiogenesis and vasculogenesis processes. For instance, research using microfluidic chips indicated that pericytes, via a mechanism involving interleukin-6, are more effective in promoting vascularization compared to stromal cells [22]. Similarly, Zhang et al. (2022) [23] demonstrated that interstitial flow (IF) significantly enhances the formation, density, and perfusability of self-organized microvascular networks (MVNs) in a microfluidic system by upregulating matrix metalloproteinase-2 (MMP-2). Validated with a brain MVN model, the strategy was projected to boost diverse organotypic MVN development for bioengineering applications. Shakeri et al. (2023) [24] have critically assessed the design features, fabrication materials, biofunctional interfaces, and key progress of various OoC models for modeling different vascular diseases (atherosclerosis, thrombosis, vascular dysfunctions, inflammation, malformation, pulmonary arterial hypertension, ischemia, etc.).

In a similar vein, tumor-on-a-chip (ToC) models have garnered significant research attention. For example, Lansche et al. (2025) [25] developed a patient-derived, vascularized ToC (vToC) model. This model revealed that cancer cells and cancer-associated fibroblasts (CAFs) induce endothelial anergy by downregulating VCAM-1 leukocyte adhesion protein and immunomodulatory chemokines, thereby offering a clinically relevant platform for tumor–CAF-immune–endothelium interaction studies. Similarly, the use of organotypic pancreatic ductal adenocarcinoma (PDAC)-on-a-chip model to understand tumor vascular invasion and tumor-blood vessel interactions revealed activin-ALK7 signaling-mediated endothelial ablation [26]. Angiogenesis chips can also be used to recreate the tumor microenvironment and study tumor growth, investigate the role of angiogenesis in cancer, and screen drugs. In the realm of cosmetology, skin-on-a-chip models are being developed to replace animal testing (e.g., the microfluidic model developed by Jusoh et al. (2019) [27] utilized the angiogenic response, stimulated by irritated keratinocytes, to predict skin irritation by chemical irritants).

The progress in vascularized microfluidic models of different structures (including heart, liver, kidney, skin, endometrium, etc.) as well as studies on neurovascular unit-on-a-chip and vascularized tumor-on-a-chip platforms has been the subject of multiple recent reviews [7, 9,13,16,17]. As an exemplary piece of evidence, Mozneb et al. (2024) [28] presented a novel microfluidic OoC that integrates human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes (CMs) (representing heart muscle, myocardium) and hiPSC-ECs (representing vasculature) in a dynamic environment with active fluid flow and rhythmic biomechanical stretch. This advanced model augmented cellular maturity (functional and genetic), mimicked physiological conditions (endothelial barrier permeability), and enabled the investigation of multi-lineage cardiotoxicity (induced by VEGFR2/PDGFR-inhibiting tyrosine kinase inhibitors (VPTKIs) (chemotherapeutic agent)). By improving the prediction of cardiotoxicity, this technology holds prospects to accelerate drug development and potentially reduce the risk of therapy-induced adverse cardiovascular events in cancer patients. In the same way, readers may go through Arslan et al. (2024)'s protocol for developing a microfluidic organ-on-chip platform that enables the generation of human induced pluripotent stem cell (hiPSC)-derived cardiac microtissues with perfusable blood vessels [29].

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At this juncture, for the illustration of the nuances and the growth in the realm, a few more exemplary studies are discussed.

[A] In a topical investigation, a microfluidic chip-based in vitro model was developed to recapitulate the early stages of human endochondral ossification, with an emphasis on vascular interactions [30]. Building on prior in vivo models using human embryonic stem cell (hESC)-derived sclerotome implanted in immunodeficient mice, the researchers developed an OoC system to simulate vascular invasion. Fibrin gel proved more effective than collagen-I gel in aligning mCherry-labeled human umbilical vein ECs (HUVECs), facilitating the formation of perfusable, vascular-like networks. Incorporating hESC-derived sclerotome and enhanced green fluorescent protein (EGFP)-expressing HUVECs produced SH (Sclerotome + HUVECs) organoids, which exhibited augmented maintenance and organization of vascular structures. Interestingly, the authors reported the migration and partial integration of the EGFP-expressing HUVECs with pre-established mCherry-HUVEC networks. Immunohistochemistry revealed spatially distinct expression of SRY-box transcription factor 9 (SOX9) in mesenchymal condensates and type I collagen in perichondrial-like regions, further validating the model's physiological relevance. The platform may be applied to understand vascular dynamics in human endochondral ossification, modeling genetic bone conditions, and drug discovery in skeletal biology. However, the limitations, including incomplete skeletal cell maturation hindering cartilage vascular invasion, unachieved intra-organoid vascular perfusion, and chip system constraints on long-term SH organoid culture, cannot be overlooked.

[B] Quintard et al. (2024) developed a novel cyclic olefin copolymer (COC)-based microfluidic platform for generating vOoC, specifically designed to overcome challenges like manual operation and PDMS material incompatibilities [14]. The system ensured stable, two-week-long perfusion at 1 μL/min via a syringe pump, allowing continuous multi-channel monitoring. A key innovation was the passive loading of organoids and hydrogels into hydrodynamic traps, which minimized shear stress and enabled the formation of interconnected, multi-organoid arrays. The platform reproducibly generated perfusable endothelial networks, recapitulating in vivo vascular patterning and achieving physiologically relevant blood flow velocities (v = 100–7500 μm/s) and shear rates ($\dot{\gamma}$ = 25–2000 s^{-1}). Crucially, successful anastomosis between a HUVEC endothelial bed and human blood vessel organoid capillaries demonstrated intravascular perfusion. This study further attested flow and vascularization-mediated enhancement of pancreatic islet spheroid function: a high glucose challenge upscaled insulin secretion, but a significantly higher stimulation index was observed only with both fluid flow and an integrated HUVEC vascular bed, indicating a synergistic effect critical for an enhanced functional β-cell niche (Figure 1). Current challenges include significant cell loss during hydrogel encapsulation and difficulties in precisely adjusting gel layer thickness, impacting scalability and efficiency.

[C] Likewise, a novel microfluidic chip was introduced by Oliveira et al. (2024) to achieve sequential in vivo organoid vascularization and subsequent successful transition to in vitro culture, critical for sophisticated tissue modeling [31]. The study evaluated two PDMS chip prototypes using the chicken chorioallantoic membrane (CAM) model. Both designs utilized a standard three-channel architecture, with the central channel, loaded with mouse embryonic kidneys (MEKs) and hydrogel for vascularization, flanked by lateral channels for medium perfusion (Figure 2A,B). Prototype A enabled initial infiltration of chicken-derived vessels into the hydrogel; however, its design impeded successful chip detachment, making the vascularized tissue unsuitable for further in vitro analysis. This limitation was overcome by Prototype B, which incorporated a refined membrane with 30 μm pores. This modification facilitated robust vessel ingrowth while preserving hydrogel and MEK structural integrity during chip detachment from CAM, enabling reliable ex ovo vascularization and seamless transfer to in vitro culture for longitudinal studies. The addition of vascular endothelial growth factor (VEGF) within the hydrogel, in combination with endogenous VEGF production by embryonic kidneys, effectively directed angiogenic sprouting from the CAM into the chip. Vascularization occurred within 3–5 days, with functional blood flow confirmed. The critical transition step to in vitro culture required meticulous removal of residual CAM tissue, avoiding medium leakage. Comparative analysis of vascular development under different conditions (Figure 2C) demonstrated the efficacy of the novel system. While MEKs cultured exclusively in vitro without CAM transplantation showed a significant reduction in their vascular network, samples vascularized on the CAM successfully integrated chicken-derived capillaries with pre-existing mouse vessels, restoring circulation and promoting continued vascular development. Crucially, samples vascularized on the CAM using Prototype B and subsequently transferred to in vitro conditions, maintained intact mouse-derived blood vessels forming a robust vascular network, with minimal degradation of the kidney tissue. This work established a highly functional platform for initiating in vivo organoid vascularization and effectively sustaining it in vitro, thereby advancing dynamic tissue modeling.

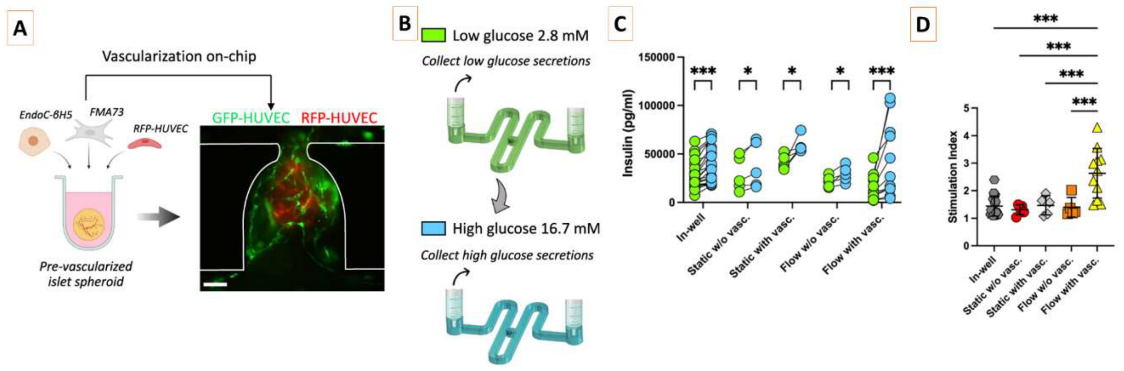

Figure 1: [**A**] Generation of pre-vascularized pancreatic islet spheroids {involving the use of EndoC-βH5 (Human primary β cells), FMA73 (Primary human fibroblasts), and RFP-labelled HUVEC} for on-chip culture within an HUVEC endothelial bed and under flow conditions. Scale bar, 100 μm. [**B**] Schematic diagram of glucose stimulation and collection of secretions performed on-chip. Comparison of the [**C**] insulin secretions and [**D**] stimulation index of the pancreatic islet spheroids between the various culture conditions. Each point on the graph represents the stimulation index of one pancreatic islet obtained from the measurement of low and high glucose stimulation. Data represents mean ± s.d. Statistical significance was attributed to values of $P < 0.05$ as determined by unpaired t test (two-tailed). * $P < 0.05$ ($P = 0.017$ (static w/o vasc.), $P = 0.029$ (static with vasc.), $P = 0.037$ (flow without vasc.)), *** $P < 0.001$. (Reproduced from Quintard et al. (2024) [14] under the provisions of http://creativecommons.org/licenses/by-nc-nd/4.0/. Copyright © 2024, The Author(s). Access date: 10 May 2025).

[D] Cerebral neovascularization is intrinsically regulated by metabolic demands and profoundly influences neurodevelopmental processes such as neuronal plasticity, gliogenesis, and neurogenesis [32]. Reciprocally, molecular factors from neurons and surrounding cells dictate EC behavior and angiogenic potential. Given the intricate and incompletely understood nature of cerebral vascularization, alongside challenges in its in vitro recapitulation, Shaji et al. (2024) [32] aimed to delineate the molecular mechanisms governing neurovascular crosstalk crucial for establishing functional in vitro vascularized cerebral organoid (CO) systems. Their microfluidic CO-vascular bed (CO-VB) co-culture system, combined with transcriptomic profiling of the CO's outermost cell layer on a preformed vascular bed, revealed highly orchestrated regulation of numerous pro-angiogenic factors and their downstream effectors. The platform sustained CO lineage integrity and pre-formed VB integrity for up to 10 days, though the angiogenic response within the CO attenuated after day 7. Transcriptome analyses indicated a temporal regulation of angiogenic potential, with early activation (day 31) and subsequent suppression (day 35) of key pro-angiogenic genes, particularly VEGFA, and catenin beta-1 (CTNNB1). Furthermore, Cysteine-Rich Angiogenic Inducer 61 (CYR61) and Hepatoma-Derived Growth Factor (HDGF) were found to augment vascular sprout formation in the CO, suggesting their functional involvement in central nervous system (CNS) angiogenesis. Although, the study provided crucial insights for advancing in vitro brain vascularization strategies, the use of non-brain specific HUVECs and human lung fibroblasts (hLFs) seemed to potentially limit the fidelity of the neurovascular mimicry vis-à-vis the specialized structures of the brain vasculature- the blood brain barrier (BBB) with pericytes and other stromal cells. Future research could focus on incorporating brain-specific microvascular endothelial cells (ECs) or ECs derived from induced pluripotent stem cells (iPSCs), utilizing perfusion-based culture systems, and employing spatial omics or single-cell sequencing approaches to more comprehensively characterize the dynamic vascularization landscape in cerebral organoids (COs).

[E] Casademont-Roca et al. (2025) [33] recently introduced the first spongy urethra-on-a-chip model, based on the F300R microfluidic device and a rocking system. A significant achievement was the successful formation of microvessel-like structures by co-culturing HUVECs with pericytes within 3.5% gelatin-transglutaminase hydrogels. Crucially, even a low fluid shear stress of 0.049 dyne/cm^2 was sufficient to promote a confluent urethral epithelial monolayer with organized tight junctions under dynamic conditions. These vasculogenic hydrogels also demonstrated the capacity to support the infiltration and proliferation of chicken ECs in the CAM assay. While promising, limitations include the use of mixed species cells, a fluid shear stress significantly lower than physiological levels, and challenges in hydrogel cross-linking. Despite these, evinced by the successful recapitulation of both the epithelial and vascular compartments under dynamic flow, the platform is envisaged to offer a valuable tool for investigating urethral stricture disease mechanisms and preclinical drug screening.

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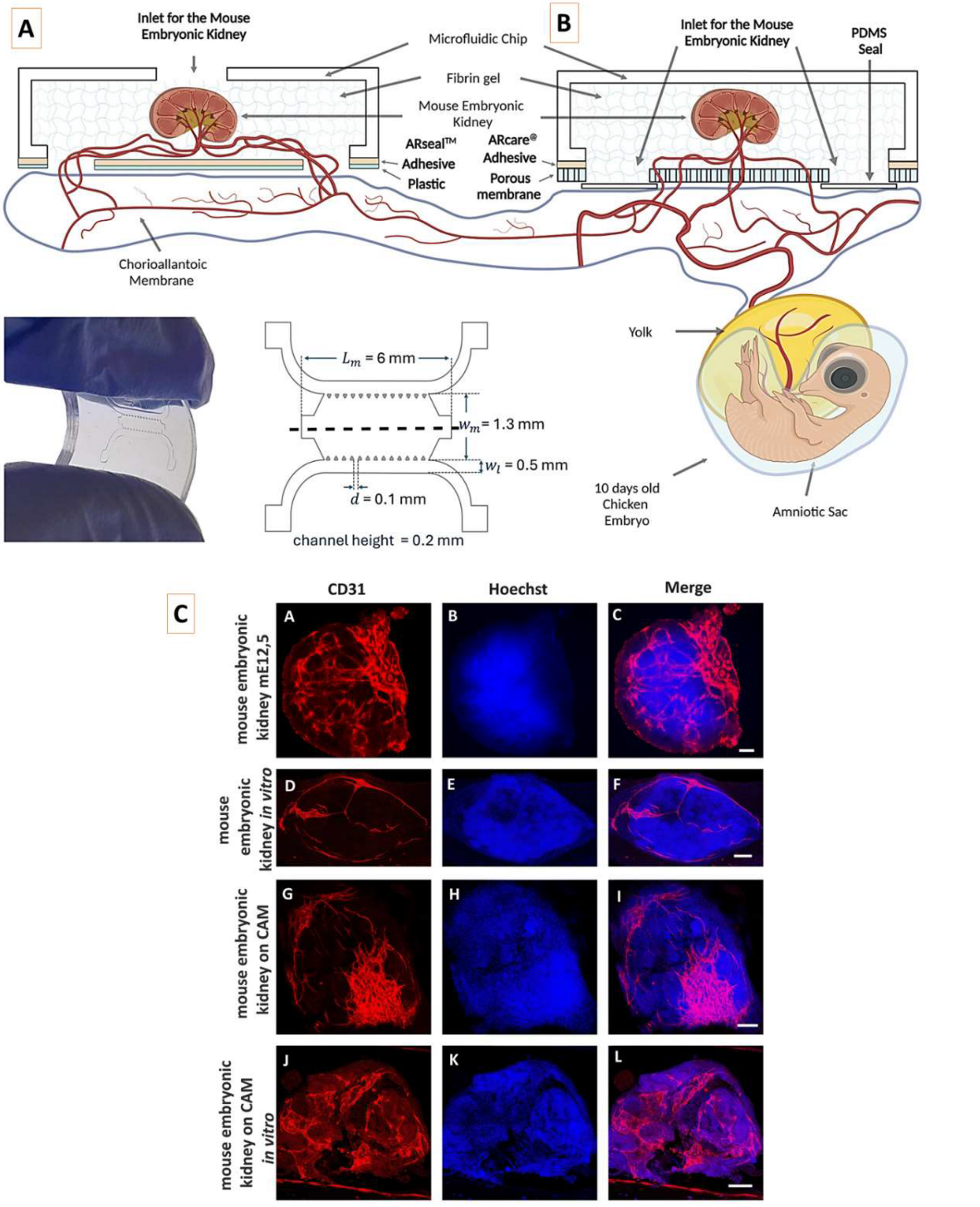

Figure 2: [**A**,**B**] Schematic representation of the microfluidic chip on a CAM. Prototypes A and B are shown in lateral and top views with dimensions. The mouse embryonic kidney is schematically vascularized by capillaries originating from the chick embryo chorioallantoic membrane (CAM). Created with https://BioRender.com. [**C**] Detection of the blood vessels in mouse embryonic kidneys in different variants of the experiment. Positive control: intact mouse embryonic kidney dissected from E12,5 embryo (A–C). Negative control: mouse embryonic kidney dissected from an E12,5 embryo, cultivated 5 days in vitro (D–F). In vivo experiment. Mouse embryonic kidney E12,5 was vascularized on a chicken embryo CAM for 4–5 days (G–I). In vivo experiment after continuation in vitro. Mouse embryonic kidney E12,5 was vascularized on the chicken CAM for 4–5 days, and then cultivated for 5 days under in vitro conditions (J–L). CD31 was used as an endothelial marker (red) and Hoechst as a nuclear marker (blue). Scale bars = 50 μm. (Reproduced from Oliveira et al. (2024) [31] under the provisions of Creative Commons Attribution 3.0 (Unported) License. Copyright © The Royal Society of Chemistry 2024. Access date: 11 May 2025).

[F] To achieve in vitro tissue grafting, Bonanini et al. (2022) [34] designed an open-top microfluidic platform, facilitating the direct introduction of target tissue via manual or robotic pipetting. The system's 384-well microtiter plate integrates 64 microfluidic chips, each supporting angiogenesis and microvascular network formation between two main lateral vessels. They demonstrated the vascularization and subsequent perfusion of liver tissue constructs (Figure 3). A key validation was achieved by mimicking veno-occlusive disease (VOD) through azathioprine exposure, which induced a measurable loss of network perfusability and localized microvascular cell death, providing a functional readout for disease modeling. Critical comparison of liver tissue constructs revealed distinct outcomes: both liver spheroids and prevascularized spheroids achieved anastomoses, but prevascularized spheroids displayed a twofold higher success rate in establishing perfusable cultures, underscoring the crucial advantage of endogenous endothelium for functional network formation. In contrast, liver organoids did not reliably induce anastomosis or perfusability. The authors critically hypothesized that this limitation stemmed from the growth factor-rich organoid culture medium and potential hindrance from the collagen-Matrigel interface, impeding vascular elongation and interconnection. The platform's broad applicability extends beyond liver tissue, envisioned for vascularizing other organoids (e.g., brain, kidney) and tumor tissues, offering a more efficient alternative to time-consuming and variable patient-derived xenograft models. Future research directions could involve incorporating specialized cells such as Liver Sinusoidal ECs (LSECs) and other non-parenchymal cells (e.g., stellate and Kupffer cells), to enhance physiological fidelity for complex disease modeling.

[G] Vasudevan et al. (2025) [35] introduced the innovative OrganiX™ microfluidic platform for enhanced in vitro vascularized tumor modeling and therapeutic discovery (Figure 4). The 24-well system with removable inserts supports high-throughput analyses and diverse downstream assays. Its substantial capacity to accommodate tumors up to 2 mm in diameter allows for the faithful recapitulation of complex tumor biology, including the formation of hypoxic or necrotic cores and dense vascular networks, which represents a significant advancement over previous models. The platform features a user-friendly, gravity-driven flow system. Using HepG2 and Hep3b liver cancer cells, it generated optimized tumor spheroids (>500 μm) within a complex tumor microenvironment (TME) with self-assembled, perfusable vascular networks exhibiting *in vivo*-like permeability (~1.8 $\times$ 10^{-7} cm/s), making it suitable for drug delivery investigations. The model successfully mimicked key determinants of therapeutic efficacy: a hypoxic core, extracellular matrix, and functional vasculature. Vascularization promoted tumor proliferation and reduced hypoxia. Differential responses to sorafenib (a tyrosine kinase inhibitor used in patients with hepatocellular carcinoma (HCC)), across tumor subtypes, mirrored clinical heterogeneity of treatment outcomes, highlighting complex resistance mechanisms, attributable to tumor-ECM interactions. Furthermore, the model elucidated the paradoxical role of tumor vasculature in $CD133^{+}$ chimeric antigen receptor T-cell (CAR-T) cell therapy against HCC: while the tumor endothelium acted as a physical barrier to T-cells' infiltration, the few successfully infiltrated cells retained potent cytotoxic function and exhibited reduced exhaustion markers within the TME. Spatial profiling of the tumor microenvironment uncovered specific activation and exhaustion signatures in the engineered T-cells, OrganiX™, thus, has been projected as a powerful, controlled in vitro tool to dissect TME mechanisms influencing therapy, complementing in vivo studies and accelerating personalized therapeutic development.

4. Key Challenges, Prospective Solutions, and Future Research Avenues

Microfluidics-assisted vOoC platforms have demonstrated immense potential to revolutionize biomedical research. However, the successful realization of this prospect necessitates overcoming a multitude of intricate engineering, biological, and translational challenges.

A primary set of hurdles revolves around device fabrication and engineering. Achieving precise microchannel dimensions for consistent fluid flow and optimal cellular behavior remains challenging, especially when incorporating multiple components such as sensors, valves, and pumps. Air bubble formation within microdevices consistently disrupts function and harms cells, demanding robust priming methods and automated detection/removal systems [18]. The development process itself is time-consuming, often spanning days for PDMS devices to weeks for gel-based systems [18]. Moreover, ensuring reproducibility across devices is critical yet challenging due to inherent sensitivity to environmental fluctuations and equipment malfunctions. Material limitations, including drug absorption by common polymers like PDMS, biocompatibility issues, and biofouling, also demand novel solutions. Maintaining precise flow control for nutrient/oxygen transport and shear stress is crucial but can be difficult to achieve dynamically [24], as is the uniform distribution of high densities of ECs in small-diameter microchannels [18]. High scattering of turbid tissues in vOoC models limits optical imaging depth [19]. Moreover, scaling up production for high-throughput applica-

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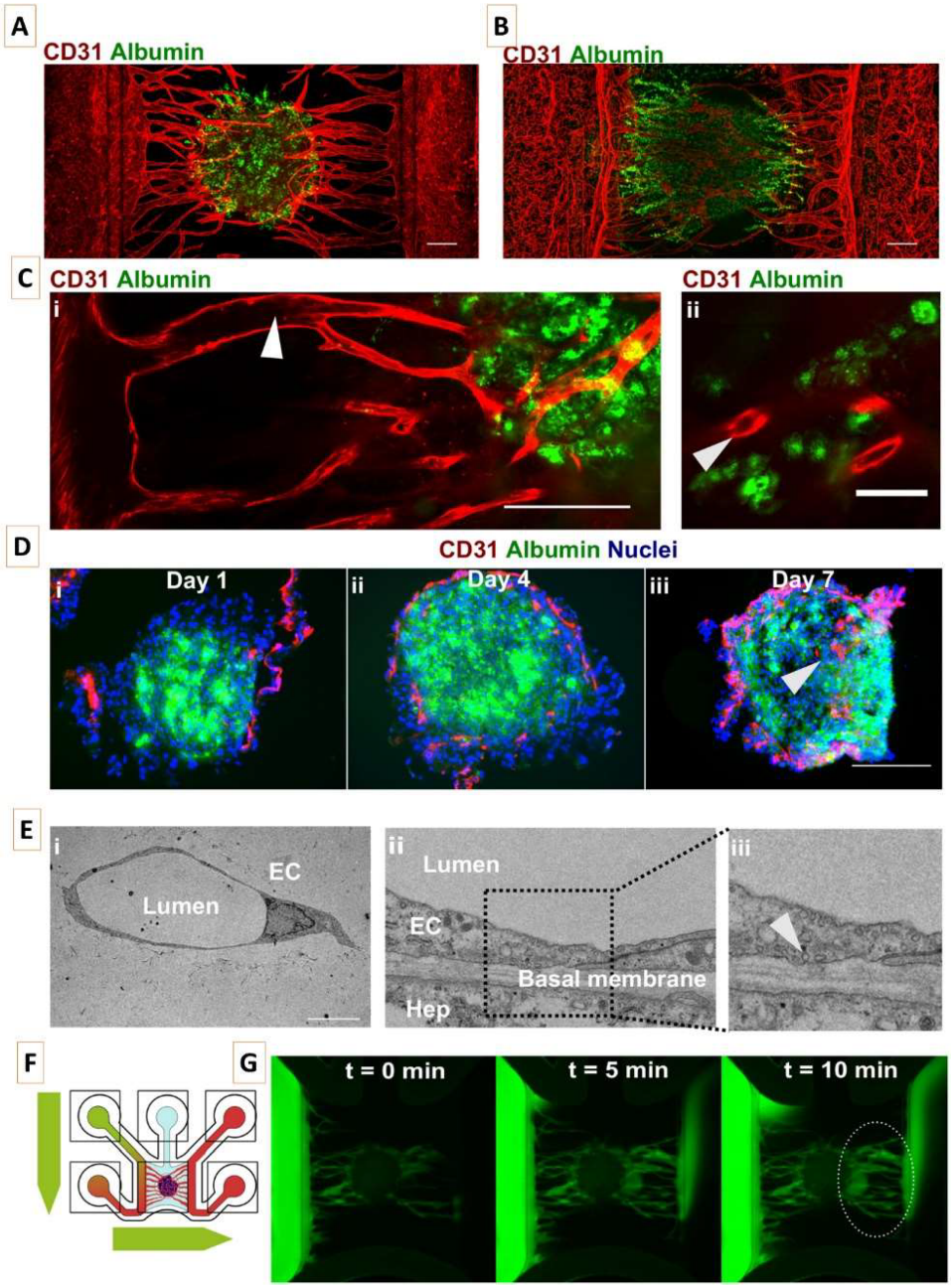

Figure 3: ECs penetrate hepatic microtissues, forming lumenized, perfusable microvasculature. [**A**] Maximum intensity projection of hepatocyte spheroid co-cultured with microvessels for 7 days and stained against albumin (green) and CD31 (red). Scale bar: 200 μm. [**B**] Maximum intensity projection of hepatocyte organoids co-cultured with microvessels for 21 days and stained against albumin (green) and CD31 (red). Scale bar: 200 μm. [**C**] Single-plane confocal images of hepatocyte spheroid co-cultured with microvessels for 7 days and stained against albumin (green) and CD31 (red). Lumenized spaces are observable in the vessels connecting the main tubes to the hepatocyte spheroid (**I**, white arrow) and in close proximity to hepatocytes (**II**, white arrow). Scale bar: 200 μm (i) and 50 μm (ii). [**D**] Cryo-sections of spheroids co-cultured for 1 (i), 4 (ii), and 7 (iii) days on microvascular beds stained against albumin (green), CD31 (red), and nuclei (blue). The white arrow indicates penetration of CD31 + ECs inside the hepatocyte spheroid. Scale bar: 200 μm. [**E**] EM images of sections of hepatocyte organoids co-cultured with microvessels for 7 days, indicating ECs with distinct lumen (i) and vascular-hepatic contact point (ii) with intracellular pinocytic vesicles (iii, white arrow). Scale bar: 10 μm. [**F**] Schematic depiction of flow through the chip to assess the perfusability of vascularized liver cultures using 150-kDa FITC-labeled dextran. [**G**] Over-time fluorescence microscopy images of 150-kDa FITC-labeled dextran perfusing a hepatocyte spheroid co-cultured for 7 days with microvessels. Dotted ellipse indicates FITC-labeled dextran emerging from the spheroid and flowing in the lateral perfusion channel (Reproduced from Bonanini et al. (2022) [34] under the provisions of http://creativecommons.org/licenses/by/4.0/, Copyright © 2022, The Author(s). Access date: 11 May 2025).

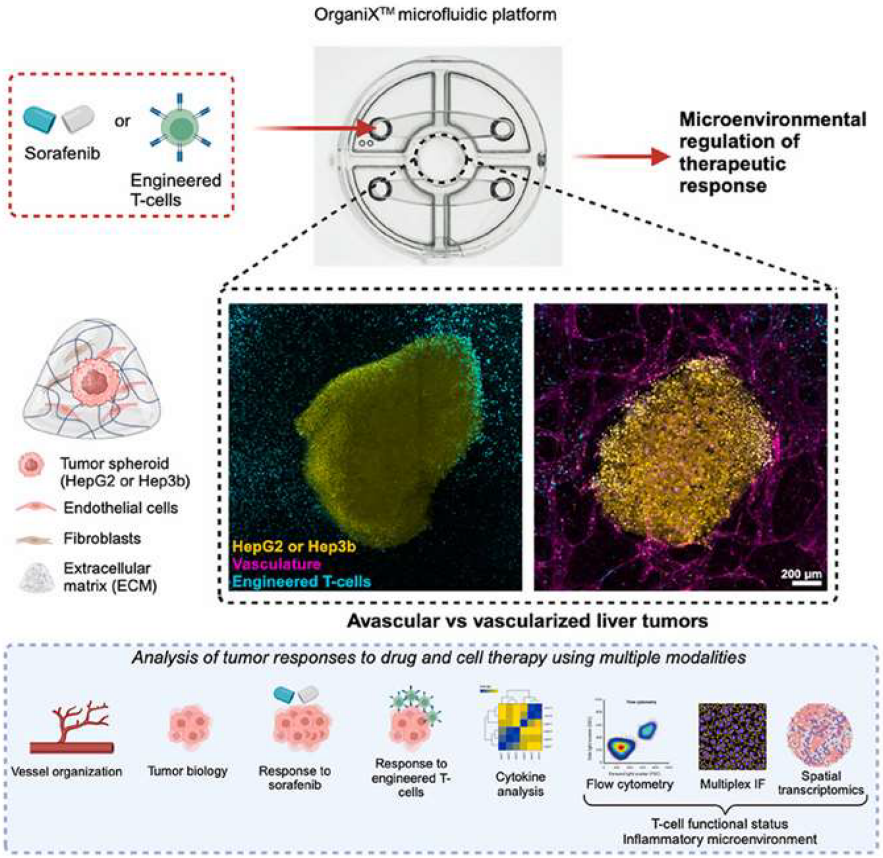

Figure 4: Representation of the vascularized human liver tumor model based on a microfluidic platform, designed to test both drug and cell-based therapies (Reproduced from Vasudevan et al. (2025) [35] under the provisions of https://creativecommons.org/licenses/by/4.0/ Copyright © 2025 The Authors. Published by Elsevier Ltd. Access date: 11 May 2025).

tions and integrating these systems into multi-organ platforms introduces significant cost and time constraints.

Beyond engineering, biological mimicry presents profound challenges. Replicating the intricate branching and hierarchical structure of in vivo vascular networks, complete with dynamic physiological conditions like pulsatile flow and the precise incorporation of diverse cell types, is a complex task. A major drawback is the significant underrepresentation of the immune system in most OoCs, lacking key immune organs and diverse immune cell populations, which severely limits accurate modeling of systemic infections, inflammation, and hemostatic issues [11]. Furthermore, the typically short lifespan (14–21 days) of OoC cultures restricts the study of chronic diseases and long-term vascular remodeling, as cells in artificial environments can eventually regress or undergo apoptosis. Ensuring physiologically relevant barrier function and using appropriate organ-specific ECs are also critical for high-fidelity modeling.

Addressing these complex challenges requires multifaceted prospective solutions and future research themes. Advancements in fabrication involve standardized modular platforms, advanced 3D bioprinting, and AI-guided design optimization, alongside rapid prototyping techniques. Automated bubble management and robust fluidic systems with real-time feedback control are essential for reproducibility. Research into novel biomaterials, dynamic surface functionalization, and integrated biosensors is crucial for improved biocompatibility and real-time monitoring of physiological parameters. To enhance biological fidelity, strategies include incorporating organoid-derived immune compartments, expanding co-cultures with diverse immune cell populations, harnessing stem cell biology for extended viability, and developing improved ECM mimics and dynamic culture systems. Overcoming the limitations in vascular complexity replication necessitates advanced biomanufacturing techniques (e.g., two-photon polymerization) and specialized cell seeding methods. For example, the 3D soft microfluidic strategy by Grebenyuk et al. (2023) [36] relied on a 3D-printable, 2-photon-polymerizable hydrogel to precisely print microvessels below living tissues' diffusion

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limit. This enabled the creation of large, viable, and proliferative engineered tissues with complex morphogenesis over long-term culture, preventing hypoxia and necrosis. Similarly, employing tissue optical clearing (TOC) with efficient large-scale data analysis methods can assist in comprehensive 3D visualization of vOoCs [19].

Ultimately, standardizing validation methods, miniaturizing analytical platforms, and creating unified data protocols are vital for translational utility. The collective efforts, bridging bioengineering, material science, immunology, and computational modeling, are poised to transform these promising platforms from laboratory innovations into clinically and industrially relevant tools for drug discovery, disease modeling, and personalized medicine. Of note, Gaillard et al. (2023) [37] proposed a typology of organoid technology from a philosophical and ethical standpoint. Its core argument is that different types of organoids possess distinct emerging properties, leading to varied ethical implications. By classifying these biotechnological constructs, the authors have attempted to clarify the complex ethical landscape surrounding organoid research, addressing both traditional ethical concerns (like informed consent) that are amplified by organoids' novel nature, and new challenges arising from properties such as potential sentience (neural organoids) or organismal capabilities (embryo models).

5. Conclusions

A brief overview of the strategies to harness microfluidics for vascularization of OoC platforms, the advantages, and key challenges of this technology, as well as a few topical reports across various biomedical/bioengineering applications were presented in this article. OoC models offer significant advantages in drug discovery and personalized medicine by reducing animal reliance and improving clinical outcome predictions. Vascularization is crucial for accurately mimicking the complex tissue microenvironment within these models, enabling researchers to study disease progression and evaluate therapeutic interventions effectively. Microfluidics plays a pivotal role in achieving vascularization within OoC models. By precisely controlling fluid flow, these devices recreate physiological conditions such as shear stress and pulsatile flow, critical for EC function and vascular network formation. Furthermore, microfluidic platforms facilitate the generation of growth factor gradients, replicating their spatial distribution in vivo and enabling precise investigation of angiogenesis. Current vascular chips primarily focus on specific organs, while the development of universal platforms is a critical next step. Challenges are encountered in integrating complex vascular networks, encompassing fabrication complexities, ensuring long-term patency and stability, and faithfully replicating tissue-specific microenvironments. Advancements in vascularization technology are envisaged to drive the development of interconnected OoC systems. This requires interdisciplinary collaboration among experts in biomaterials, microfluidics, and cell biology. Universal chips will significantly impact personalized medicine, particularly in drug screening for individual tumors, and streamline clinical trials. Such progress can pave the way towards the creation of human-on-a-chip models, which hold immense potential for revolutionizing organ transplantation.

List of Abbreviations

AI	Artificial Intelligence
BBB	Blood Brain Barrier
CAFs	Cancer-Associated Fibroblasts
CAM	Chorioallantoic Membrane
CAR-T	Chimeric Antigen Receptor T-cell
CMs	Cardiomyocytes
CNS	Central Nervous System
CO	Cerebral Organoid
COC	Cyclic Olefin Copolymer
CTNNB1	Catenin beta-1
CYR61	Cysteine-Rich Angiogenic Inducer 61
EC	Endothelial Cells
ECM	Extracellular Matrix
EGFP	Enhanced Green Fluorescent Protein
HCC	Hepatocellular Carcinoma
HDGF	Hepatoma-Derived Growth Factor
hESC	Human Embryonic Stem Cell
hiPSC	Human Induced Pluripotent Stem Cell
hLFs	Human Lung Fibroblasts
HUVECs	Human Umbilical Vein ECs
IF	Interstitial Flow
LSECs	Liver Sinusoidal Endothelial Cells
MEKs	Mouse Embryonic Kidneys
MMP-2	Matrix Metalloproteinase-2
MVN	Microvascular Networks
OoC	Organoid-on-a-Chip
PDAC	Pancreatic Ductal Adenocarcinoma
PDMS	Polydimethylsiloxane
SH	Sclerotome + HUVECs
SOX-9	SRY-Box Transcription Factor 9
TME	Tumor Microenvironment
TOC	Tissue Optical Clearing
ToC	Tumor-on-a-Chip
VB	Vascular Bed
VCAM-1	Vascular Cell Adhesion Molecule 1
VEGFA	Vascular Endothelial Growth Factor A
VoC	Vessel-on-a-Chip/Vasculature-on-a-Chip

VOD	Veno-Occlusive Disease
vOoC	Vascularized Organoid-on-a-Chip
VPTKIs	VEGFR2/PDGFR-Inhibiting Tyrosine Kinase Inhibitors
vToC	Vascularized Tumor-on-a-Chip

Author Contributions

The author conducted the literature review and was responsible for writing, reviewing, and editing the manuscript.

Availability of Data and Materials

Not applicable.

Consent for Publication

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Conflicts of Interest

The author declares that he has no conflicts of interest.

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