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The Vascular Niche in Next Generation Microphysiological Systems

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The vasculature is a multifunctional organ critical for life; In addition to facilitating the distribution of oxygen and nutrients across tissues of the body, blood vessels play a key role in the active transport of immune cells, metabolites, and therapeutics, and in the regulation of blood pressure and the maintenance of normal body temperature. Given the diverse biological functions of the vasculature, it is not surprising that the disruption or maladaptation of the vasculature is a critical step in the progression of many diseases. While some diseases afflict the vasculature itself such as coronary artery disease¹ and Sturge-Weber Syndrome², other diseases such as cancer co-opt blood vessels to meet growing metabolic needs. As such, the vasculature has long been considered as a therapeutic target^{3–5} and a major contributor to tissue identity and function. Unfortunately, the vasculature is rarely included in *in vitro* disease models, perhaps contributing to the slowdown in approval of new drugs, despite our increased understanding of the genotypic and phenotypic basis of disease. Indeed, recent data show that the Food and Drug Administration (FDA) approves a mere 13.8% of *all* drug candidates.⁶ This pervasive clinical failure of therapeutics emphasizes the need for improved, physiologically-relevant *in vitro* models of human disease for drug screening, and we make the argument that incorporating the vasculature into these models will be essential.

Current preclinical studies rely heavily on 2D *in vitro* models or murine *in vivo* models, both of which, for different reasons, fail to recapitulate vascularized human tissues and thus the behavior of drugs in the body. To address the prevailing dissonance between preclinical models and human studies, researchers have sought alternative human cell-based *in vitro*

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Competing Interests

MLE and YHC declare no competing interests. CCWH and APL have equity interests in Aracari Biosciences, Inc, which is commercializing the microfluidic devices described in this paper (Figure 2). The terms of this arrangement have been reviewed and approved by the University of California, Irvine in accordance with its conflict-of-interest policies.

platforms not only to assess novel therapeutics, but also to interrogate disease biology. There has been a growing focus on 3D culture technologies, for example, such as stem cell-derived organoids^{7,8} and patient-derived spheroids,⁹ as these better maintain *in vivo* functions in culture owing to the 3D conformation they adopt, allowing for more physiologic cell-cell and cell-extracellular matrix interactions. Unfortunately, the potential of these 3D culture techniques to yield preclinical data with a higher predictive value compared to 2D studies has yet to materialize, mostly due to issues surrounding biological and disease relevance, maintenance of tissue identity, and scalability.⁷ As such, none of these 3D *in vitro* models has, to our knowledge, been ubiquitously adopted in preclinical studies by the pharmaceutical industry or in academia, although strategies focused on developing the capacity to vascularize organoids is growing.¹⁰

Microphysiological System Platforms: A systematic approach to identify key cellular and molecular determinants driving human physiology, pathophysiology and clinical pharmacology.

Recently, microphysiological system (MPS, also known as, organ-on-a-chip or tissue chip) platforms have emerged with great promise to improve the predictive capacity of preclinical modeling and bridge the translational gap. While platforms vary in design, MPS are bioengineered *in vitro* environments containing human cells that aim to recapitulate key functional units of tissues and organs. As recently defined by the National Center for Advancing Translational Sciences (NCATS) at the National Institutes of Health (NIH), MPS platforms have three critical and defining characteristics: “the 3D nature and arrangements of the tissues on the platforms; the presence and integration of multiple cell types to reflect a more physiological balance of cells; and the presence of biomechanical forces relevant to the tissue being modeled.”¹¹ The rapid development and maturation of MPS models have been made possible by the adoption of several biomedical technologies developed over the last decade including advanced cell sourcing options, genome editing, and microfluidic techniques. In addition, the advent of single cell RNA sequencing has allowed unparalleled insights into how cells interact with each other in complex tissues. As a result, MPS are uniquely positioned to become physiologically and functionally relevant models that recapitulate several organ micro-features (multicellular composition and architecture, native ECM constituents etc.), including the ability to emulate biomechanical forces, such as flow-derived shear stress.

Much of the MPS literature published thus far has focused on demonstrating the physiological relevance of individual platforms and the proof-of-concept experiments relating to the capacity of the platforms to replace preclinical 2D culture methods. Increasing evidence is emerging detailing how these platforms not only have the capacity to work synergistically with murine models, but also how they provide an independent research tool for interrogating biological phenomena. Utilizing these human-derived systems, diverse biological advances have been made, from resolving aspects of the neuroinflammation and endothelial-to-mesenchymal transition (EndoMT) pathways, to elucidating the role of diseased endothelium in Hutchinson-Gilford progeria syndrome (HGPS).¹²⁻¹⁴ Thus, MPS platforms hold great promise for both basic science and clinical breakthroughs.

The Vascular Niche and MPS Platforms: Recapitulating developmental and regenerative programs to improve model organ fidelity and function.

A shared goal amongst many developers is the incorporation of a vascular compartment into the next iteration of MPS platforms. Inclusion of the vasculature is considered an important developmental target as blood vessels not only pervade the body's tissues providing a thoroughfare for blood borne solutes and cells, but also plays a critical role in organogenesis and organ identity. Indeed, increasing evidence is accumulating regarding the extent to which the unique molecular signatures and functional properties of tissue specific endothelial cells (ECs, the single layer of cells lining the blood vessel lumen) contribute to organ differentiation, identity and function.^{15,16} As such, the incorporation of a vascular compartment is not only pertinent to the delivery of drugs and nutrients in an *in vivo*-like fashion to the MPS parenchyma, but also to the development of tissue identity itself. In other words, the vasculature and its niche are essential components of generating physiologically-relevant tissues. Here, we define the vascular niche as the microenvironment proximal to organotypic microvasculature whereby distinct, endothelial extrinsic cues (soluble factors, exosomes, ECM deposition, etc.) actively influence organ regeneration, patterning, and homeostasis often orchestrated with or executed by a resident stem cell population (Fig. 1). Accordingly, current and the next generation of MPS platforms that incorporate a vascular component leverage several developmental and functional characteristics of the endothelium that are critical for the creation of more physiologically relevant models.

This review will discuss published platforms and present considerations for next generation MPS platforms looking to incorporate this critical constituent. First, we discuss EC sourcing, including predominant cell types currently used in the field. Next, we discuss EC mechanosensing and mechanotransduction and discuss options for extracellular matrix that supports not only the vasculature, but also contributes to organ identity. This is followed by a discussion on endothelial shear stress and interstitial flow and how this affects endothelial homeostasis and vascular network formation. Finally, we discuss how establishing the vasculature in next-generation vascularized MPS platforms will take advantage of unique organogenic processes governed by ECs, and how incorporation of a vascular niche will establish a unique human cell-derived *in vitro* model for stem cell development.

Endothelial Cell Sourcing for MPS Applications

For decades, Human Umbilical Vein Endothelial Cells (HUVECs) have served as the primary *in vitro* model for studying the physiological and pathological processes of vasculature. As HUVECs are easy to source, culture and manipulate for experimental purposes, it is unsurprising the vast majority of MPS platforms with a vascular component utilize these primary ECs. Recently, transcriptomic developments have detailed the extent to which distinct organ-specific EC expression patterns drive organotypic development and function, supporting the long-standing supposition that the vasculature is remarkably diverse.¹⁷ Based on the idea the endothelium is genetically tailored on a per-organ basis that aids overall function, the question arises whether HUVECs, widely considered as a mature EC with a phenotype unique to its resident tissue, best serve organ-specific vascularized

MPS models. In this section, we review EC sourcing and outline the benefits, detriments and various characteristics of ECs available for integration into MPS platforms.

Primary Endothelial Cells

Due to their widespread availability and relative ease of use, primary ECs dominate the study of vascular biology, and this has proven a huge advantage as they show superior physiological relevance compared to immortalized lines that rarely if ever retain the ability to make lumenized vessels in culture. Thus, the use of HUVECs and other primary ECs cells, such as HMVECs (Human Microvascular ECs), along with Endothelial Colony Forming Cell-derived ECs (ECFC-ECs) is common. The long-term use of primary ECs in the field and subsequent breadth of validation studies demonstrating the ability of primary EC to recapitulate critical aspects of vascular physiology *ex vivo* has benefited development of vascularized MPS platforms immensely. HUVECs, for instance express many important endothelial junctional proteins (e.g. ZO-1, Claudin-5),¹⁸ adhesion molecules (e.g. VE-Cadherin, PECAM-1, ICAM-1, VCAM-1)^{18,19} and selectins (e.g. E-Selectin and P-selectin).^{19,20} HUVECs also store, within Weibel-Palade bodies, von Willebrand factor (vWF), a key player in hemostasis and a well-characterized EC marker.²¹ Critically, HUVECs retain the capacity to form three-dimensional tubular structures *ex vivo* with a marked angiogenic,²² leukocyte trafficking,²³ and Nitric Oxide (NO)²⁴ production potential. Thus, as a well characterized, general model of the endothelium, it is not surprising HUVECs have been incorporated into several vascular compartments of organ-specific MPS platforms (Table 1).

With the advent of advanced transcriptomic technologies, our understanding of the extent to which organ-specific EC gene clusters establish a functionally diverse endothelium has become increasingly resolved, offering new perspectives on the relevance of incorporating HUVECs into organotypic MPS. Marcu *et al.*, for instance, investigated single-donor EC's isolated from four major organs—the heart, lung, liver, and kidneys—and demonstrated that these organ-specific ECs have unique gene expression patterns, and show distinct barrier properties, angiogenic potential, and metabolic rates.¹⁶ These isolated organ-specific ECs also have the capacity to support *in vitro* organ development and parenchyma function. When co-cultured with isolated fetal liver ECs, for example, hepatocytes exhibited markedly improved survival and supportive function in terms of albumin production. As evidence accumulates supporting the extent to which specialized ECs contribute to organ identity and function,²⁵ it is apt to consider integrating organotypic ECs into vascularized MPS over more universal models of the endothelium to best recapitulate the *in vivo* environment and organ function.

Indeed, several vascularized MPS devices that utilize organ-specific ECs see marked physiologic improvement. In comparison to HUVEC-derived *in vitro* microvasculature, Uwamori *et al.* demonstrated vascular networks generated from human brain microvascular ECs (BMECs) retain a significantly lower permeability coefficient.²⁶ Not surprisingly, this barrier-like phenotype was characterized by maintenance of tight junction protein expression, a genetic hallmark of blood-brain barrier endothelium. Thus, it may be most important to utilize organotypic ECs in MPS platforms where the microenvironment,

including the endothelium, facilitates robust organ function, which may include filtration, absorption, secretion and/or selective permeability. Lin *et al.*, for example, created a 3D vascularized renal proximal tubule MPS composed of renal cortex epithelium and glomerular microvascular ECs (GMECs) that exhibits active reabsorption via tubular–vascular exchange of solutes.²⁷ Importantly, GMECs retain a phenotype within the MPS device reminiscent of its *in vivo* state including CD31 localization at cell junctions, vWF and tight junction expression, and glycocalyx deposition on the luminal surface. With a microenvironment incorporating organotypic vasculature, this model not only exhibits selective reabsorption and transport of solutes but can also facilitate investigative studies of endothelium–epithelium cross-talk under homeostatic and disease conditions.

While the prospect of utilizing organ-specific ECs within vascularized MPS has advantages based on the potential to facilitate improved organ identity and function, in many cases access to primary, organotypic ECs may not be possible. As most available tissues are acquired from post-operation waste, biopsies for diagnostic purposes or post-mortem tissues, it would be unreasonable to suggest all MPS must incorporate organ specific ECs to be considered as physiologically relevant. While EC may well take on the “correct” phenotype if the surrounding tissue is physiologic enough to provide the appropriate cues, several strategies and technologies can be employed to engineer primary cells in higher abundance to recapitulate an organotypic phenotype, either by genetic manipulation or chemical treatment. Short-term expression of ETV2, a developmental EC transcription factor, for instance, “resets” HUVECs to a malleable progenitor state able to adopt tissue specific characteristics.²⁸ Additionally, *all-trans* retinoic acid (ATRA) has been utilized to induce a blood-brain barrier phenotype in ECs including ECFC-ECs,²⁹ a progenitor-like EC commonly isolated from umbilical cord or peripheral blood that expresses markers of the three endothelial differentiation lineages (venous, arterial, and lymphatic).^{30,31} Increasingly, ECFC-ECs, with the capacity to adopt tissue-specific EC phenotypes, are growing in popularity with MPS platform developers for their relative ease of accessibility and heightened proliferative and vasculogenic capacity (Table 1).^{30,32}

With the expansion of single cell RNA sequencing technologies, we can begin to understand how well the transcriptomes of generalized endothelium maps onto those of organotypic ECs to determine how well suited the former can be utilized in organ specific models. Lukowski *et al.* demonstrated that differentiated murine ECs originating from the aorta, for instance, have a transcriptomic profile with significant overlap to the HUVEC transcriptome.³³ The idea organotypic ECs share some transcriptomic basis with those from another organ has also been supported by recent findings; while the transcriptome of ECs derived from the brain and liver presented unique identities, ECs from other tissues (adipose, heart, and aorta) shared more significant transcriptomic similarities.²⁵ It is currently unclear to what extent functional EC properties are intrinsic, acquired during early differentiation or reprogrammed by the local environment. Thus, in some cases incorporating cells that are more mature (i.e., HUVEC, arterial EC) may be appropriate, while in others, a cell with more plasticity (i.e., ECFC-EC) might be a better choice. Indeed, MPS models may be the ideal environment in which to study these very questions.

Induced Pluripotent Stem Cell (iPSC)-derived Endothelial Cells

While incorporating primary ECs within MPS platforms is perhaps the most straightforward sourcing strategy to faithfully recapitulate the endothelium *ex vivo*, variable tissue availability, relatively poor regeneration capacity and patient-to-patient variability tends to compromise model reproducibility. The advancement of iPSC technologies can remedy these difficulties as a potential infinitely renewable and reproducible cell source. Generated by reprogramming somatic cells via forced transcription factor expression, iPSCs have the capacity to differentiate into any lineage of the three germ layers.³⁴ This unprecedented opportunity to generate ECs including those with vessel- and organ-specific properties has spurred great developments within vascular biology, benefitting developmental,^{35,36} disease^{37,38} and therapeutic modeling^{39–41} alike.

iPSC-derived EC (iPSC-EC) differentiation strategies have been extensively described.^{42,43} Briefly, most protocols direct iPSC-EC differentiation through a mesoderm lineage and then introduce endothelial specification by the addition of vascular endothelial growth factor (VEGF). Additional components such as miR-21 and transforming growth factor beta (TGF- β) amongst others can be added to increase differentiation efficiency.⁴⁴ While these chemically defined differentiation protocols are the most frequent approaches, iPSC-ECs can also be generated through the overexpression of endothelial development transcription factor ETV2.⁴⁵ Regardless of differentiation approach, pure iPSC-EC populations are captured by fluorescence-activated cell sorting (FACS) or magnetic-activated sorting (MACS) based on expression of the endothelial marker PECAM1 (CD31), and lack of hematopoietic markers, such as CD45.⁴⁶

Like their primary EC counterparts, iPSC-EC recapitulate genetic hallmarks of the endothelium, including PECAM-1 (CD31), VE-Cadherin (CD144), VEGFR2 (KDR), and vWF expression.⁴⁷ Multiple sources also report iPSC-EC exhibit typical cobblestone morphology and basic endothelial functions including limited “tube” formation on Matrigel,⁴⁸ hematological and inflammatory responses,^{49,50} and NO production.³⁶ Critically, reports show iPSC-ECs are capable of undergoing vasculogenic and angiogenic processes to form 3D conduits and vascular networks, and these have been incorporated into a handful of vascularized MPS platforms (Table 1). Unfortunately, widespread integration of iPSC-EC has been hampered by the limitations of current-generation EC differentiation techniques. Most protocols, for example, generate iPSC-ECs exhibiting deficiencies in capillary morphogenesis; in comparison to primary ECs, iPSC-ECs often make fragmented or inconsistent vascular networks.^{51,52} This suggests current iPSC-ECs protocols produce EC populations with a limited vasculogenic potential, most likely due to limited endothelial commitment and a more immature phenotype. While the molecular basis for endothelial tube formation is increasingly understood,^{53,54} the critical genetic characteristics or biophysical cues necessary for iPSC-ECs to obtain and maintain a vasculogenic capacity have yet to be determined.

Patient-derived iPSCs can also be generated, presenting a new frontier in personalized medicine. The development of iPSC-derived diseased endothelium is a particularly interesting point of vascularized MPS development as dysfunctional vasculature is a key driver in many diseases.⁵⁵ Hereditary Hemorrhagic Telangiectasia (HHT), for example, is

associated with loss-of-function mutations of Endoglin (*ENG*) or Activin A receptor like type 1 (*ACVRL1/ALK1*) and is characterized by vascular lesions, including telangiectasias and arteriovenous malformations (AVMs).⁵⁶ Patient-derived iPSC-ECs harboring genetic determinants of disease, such as those driving HHT, can not only be utilized to evaluate genetic mutations as causative factors, but also be manipulated via genomic editing to revert mutations and generate isogenic controls. For example, Zhou et al. generated iPSC-ECs from an HHT patient with a novel *ENG* missense mutation. After subcellular analysis, the authors demonstrated mutant *ENG* sequesters within the endoplasmic reticulum, affecting downstream of BMP signaling, a modulator of vascular homeostasis and remodeling. Following autologous mutant correction, partial BMP signaling was restored.⁵⁷ In a similar vein, Gu et al. corrected *BMPR2* mutations in iPSC-ECs derived from patients with familial pulmonary arterial hypertension (FPAH) and confirmed that the aberrant gene is causal for the abnormal phenotype.⁵⁸ Upon confirmation that these isogenic lines retain a vasculogenic capacity to form networks *in vitro*, the paired cell types can be integrated into MPS platforms, not only to reveal diseased endothelium genotype-phenotype associations, but also to facilitate predictive personalized medicine applications that take into account the patient's unique genetic background.

iPSC-ECs have the potential to become widely utilized within vascularized MPS platforms as they model critical aspects of the endothelium and alleviate primary EC accessibility challenges. While there has been considerable progress on subtype- and patient-specific iPSC-ECs derivation, including protocols to generate arterial and venous-like ECs,³⁶ several areas of optimization remain including maximizing efficiency, decreasing differentiation time, and generating bona fide organotypic endothelial subtypes. Moreover, iPSC-ECs as currently generated, often do not exhibit the same vasculogenic potential as either HUVECs or ECFC-EC as noted above, nor are they a homogeneous population; Paik *et al.* uncovered 4 transcriptionally unique subpopulations of iPSC-EC.⁵⁹ This heterogeneity likely plagues most iPSC-EC populations. Protocols that address these issues, however, lack uniformity, and the absence of stringent genetic and functional assessments prevents meaningful cross-comparison analysis between research groups. The problem of increasingly divergent differentiation protocols utilizing variable quality control measures was recently exemplified in a transcriptomic study of iPSC-BMECs that revealed how a widely-adopted iPSC-to-brain EC differentiation protocol⁶⁰⁻⁶² actually generates a homogenous *epithelial* cell population.⁶³ Papers that have used these cells in the formation of blood-brain barrier models will need to be re-evaluated.

Endothelial Cell Sourcing Outlook

To optimally recapitulate the endothelial compartment, integrating ECs into MPS systems relies on fit-for-use modeling decisions based on the unique MPS microenvironment, the capacity to generate or harvest organ-specific ECs and the hypothesis in question. Currently, HUVECs dominate MPS development. With the progression of transcriptomic analysis detailing the extent of endothelial diversity and the improvement of iPSC technologies, it is likely the next generation of MPS devices will primarily utilize organotypic and iPSC-ECs to improve organ fidelity. Indeed, advanced iPSC protocols are maturing, including recent approaches to generate specific endothelial lineages from iPSC derived from ECFC-

ECs.⁶⁴ This approach suggests iPSC-ECs may retain an endothelial epigenetic memory and maintain an endothelial phenotype better than ECs generated from fibroblast-derived iPSC. Ultimately, the expanding use of iPSC technology for the generation of EC, and other cells, calls for the generation of standardized protocols with stringent, agreed-upon functional and genetic validation.

Endothelial Extracellular Matrix: Vasculogenesis, Homeostasis, and Repair

The ECM is a multifunctional biological scaffold that not only provides physical support, but also contributes to tissue homeostasis via its surface topology, surface ligand landscape and composition.⁶⁵ This non-cellular meshwork is composed of a multitude of proteins and proteoglycans and delivers critical biochemical and biomechanical cues to resident cells that are essential for organogenesis, homeostasis, angiogenesis and tissue repair.⁶⁶ Thus, it is no surprise the ECM has long been used as a substrate to promote functional tissue reconstruction in clinical regenerative medicine, including esophageal repair,⁶⁷ cartilage and bone regeneration,⁶⁸ and musculotendinous tissue repair.⁶⁹ These methods to restore tissue functionality involve the delivery of constructive cues and modulation of the microenvironment through ECM signaling. Accordingly, understanding the crosstalk between cells and their surrounding ECM will offer new strategies on how to fine-tune gene expression and cell phenotype within MPS platforms. A more robust and functionally relevant model can be achieved by taking advantage of the distinct ECM-cell receptor pairings that trigger intracellular signal events and cellular responses, including pro-proliferative and migratory EC phenotypes.^{70–72} Moreover, studies demonstrate ECM composition (the scaffolding proteins and embedded growth factors) has a high degree of tissue and organ specificity, which plays a critical role in tissue and organ morphogenesis.⁷³ From this perspective, understanding how ECM influences EC behavior and organ identity is key to generating physiologically relevant and organ-specific vascularized MPS. In the following paragraphs, we briefly discuss how ECs sense and respond to chemical and mechanical cues of the ECM, what MPS scaffolding substrates are currently utilized and offer suggestions on incorporating organ-specific ECM in next-generation MPS platforms.

Endothelial Cells and Mechanosensing

The ECM is composed of various biopolymers and specialized macromolecules, including collagen, elastin, adhesion proteins (e.g., laminins and fibronectin), hyaluronans, and proteoglycans.⁷⁴ Together, these constituents work in concert to support tissues and organs; while collagen and elastin act as a scaffold providing mechanical support to resident cells, hyaluronans and proteoglycans coordinate to fill the empty spaces between fibrous structures and promote water retention within the tissue.⁷⁴ Mechanosensing describes the ability of cells to sense and integrate mechanical cues from their matrix-based microenvironment. Receptor-mediated mechanosensing is initiated via the ligation of a distinct set of cellular surface receptors and ECM pairings. This coupling results in the transmission and activation of intracellular signaling cascades including those involved in cell cycle control, migration, and matrix remodeling. The integrin family, which represents the principal cellular receptors cells use to attach to the ECM, for example, consists of eighteen α -subunits and eight β -subunits, which join into 24 combinations.⁷⁵ This subunit diversity allows for the high

degree of integrin-ECM specificity necessary for determining behavior of distinct cellular subsets.

The instructive role of ECM in endothelial homeostasis and angiogenesis

While its scaffolding function suggests a rigid-like structure, the ECM is a malleable and highly dynamic network. The constant remodeling of the ECM governed by deposition-degradation dynamics actively influences endothelial proliferative and migratory processes necessary for angiogenesis. Immobilized within the matrix of many tissues, for instance, are angiogenic cytokines, including vascular endothelial growth factor (VEGF), insulin like growth factor (IGF), fibroblast growth factor (FGF), transforming growth factor- β 1 (TGF- β 1) and hepatocyte growth factor (HGF). Upon chemical modification or enzymatic degradation of the ECM, previously sequestered factors generate instructive chemical gradients, including those necessary to induce pro-proliferative and chemotactic phenotypes. Small-molecular-weight peptides derived from degraded ECM also play a role in the attraction of ECs to remodeling sites during physiological processes such as organogenesis and wound healing.⁷⁶ Furthermore, mechanical cues generated by the ECM topography can also influence vascular remodeling in a process known as haptotaxis.⁷⁷ Haptotaxis refers to EC movement in response to gradients of ECM components that are governed by ECM-integrin interactions. HMVECs, for example, show haptotactic migration in response to gradients of fibronectin.⁷⁸

Current ECM Considerations for Vascularized MPS Platforms

Indeed, the ECM not only provides a scaffold essential for maintaining the organization of vasculature, but also the anchorage necessary for endothelial morphogenesis, proliferation, migration, survival, and homeostasis.^{73,77} Unsurprisingly, ECs in 3D cultures retain more faithful genotypic and phenotypic identities *ex vivo* than do the same cells grown in 2D.⁷⁹ While the geometric intricacies of vascularized MPS vary, capillary-like structures are primarily generated *in vitro* via three engineering strategies – self-assembled, pre-patterned, or 3D-bioprinted vasculature – with each method owning a particular set of strengths and weaknesses. Self-assembled vascularized MPS platforms, for example, recapitulate *de novo* vasculature formation *in vitro* and are thus a strong model of vascular biology. Sourcing ECs that maintain a strong vasculogenic potential *ex vivo*, however, is required, and in general, a hierarchical vasculature is hard to create. Conversely, pre-patterned and 3D-bioprinted vascularized MPS devices form fully perfusable vasculature with predefined dimensions and controlled configurations, but vessel size is largely limited by the resolution and size of the mold or nozzle, resulting in lumen diameters of 100 μ m or more.^{10,80} In addition, in many cases these vessels cannot, or do not, remodel in response to external cues. Regarding ECM selection for each vascularized MPS engineering technique, while pre-patterned vasculature MPS platforms focus on utilizing ECM micro-architectures to adhere ECs onto inert biomaterials, self-assembled and bioprinted MPS platforms seek to provide mechanical support for *de novo* vascular formation. Thus, scaffolding selection and subsequent deposition techniques necessary to generate ECM-laden platforms are largely dependent on MPS microfabrication approach.

Self-Assembled Vascularized MPS Platforms

Self-assembled vascularized MPS platforms encompass those that utilize the intrinsic capacity of ECs to form capillary-like structures within biocompatible matrices (Fig. 2A).^{81–83} This requires a malleable microenvironment that mechanically supports EC engraftment and subsequent lumenization. While several extracellular matrix proteins, including SPARC, β -IgH3 and IGFBP7, have been shown to be critical for lumen formation,⁸⁴ matrix contributions during capillary morphogenesis remains less resolved. Much of our current understanding regarding matrix-lumen dynamics derives from earlier studies in non-perfusible 3D culture that established the basic parameters for self-assembly of vessel-like structures, including a bead-based angiogenic sprouting model^{22,85} and a model of human capillary tube formation embedded within hydrogels.⁸⁶

Hydrogels mimic ECM microstructures with the capacity to retain water and facilitate nutrient and metabolite transport. Critically, hydrogels have several tunable characteristics including permeability and viscoelasticity, while advanced temperature-⁸⁷ and light⁸⁸-responsive hydrogels permit dynamic cell culture. As vasculogenic and angiogenic processes are necessary to form capillary-like networks *in vitro*, promoting specific EC-ECM interactions is especially critical for vascularized MPS platforms that are not pre-patterned. Thus, to support self-assembled vascularized MPS platforms, a composite matrix of hydrogel supplemented with additional ECM proteins is commonly used.

Collagen I hydrogels—Collagen I, an ECM protein that naturally assembles into a fibrous hydrogel, is abundantly present in many tissues. Several vascularized MPS platforms incorporate purified collagen I,^{89–92} as this hydrogel is amenable to changes in stiffness via alterations in concentration⁹³ or gelation temperature⁹⁴ and promotes endothelial survival and angiogenic processes.⁹⁵ When type I collagen binds $\alpha 1\beta 1$ or $\alpha 2\beta 1$ integrins (amongst many), the mitogen-activated protein (MAP) kinase cascade is triggered, which promotes EC viability and angiogenesis.^{96,97} Thus, collagen I not only promotes cell association within MPS platforms and its biomaterials, but also mimics the physiological cues supporting ECs and vascular network formation.

Besides EC-collagen signaling cascades that benefit angiogenesis, the ability to inject collagen I directly into microfluidic channels makes it useful for MPS applications. As a thermo- and pH-responsive polymer, collagen I is stable in acidic solutions at low temperatures and is capable of crosslinking below 37 °C and at neutral pH.⁹⁸ Because of these properties, it is easy to control collagen crosslinking after injection without the need for a catalyst. Moreover, tuning the concentration of collagen can optimize the growth of different types of cells. Adriani *et al.* presented a co-culture microfluidic platform, incorporating neurons, astrocytes, and cerebral ECs, to create a blood-brain barrier model.⁹⁹ To optimize the hydrogel for each cell type, they tuned the concentration of collagen I and found that increasing the concentration to 7 mg/ml was optimal for astrocytes, while still avoiding gel contraction, whereas 2.5 mg/mL was most suitable for neuron survival.

Fibrin hydrogels—Fibrin gel, another popular hydrogel employed in MPS platforms, exhibits excellent biocompatibility, promotes cell attachment, and can be remodeled by

ECs.^{100–103} Fibrinogen (the precursor to fibrin) self-assembles into a polymer network upon mixing with thrombin, which constitutes the basis of blood clotting. Thus, fibrin gels can “cure” *in situ* and is a suitable material to inject suspended cells into microfluidic channels.¹⁰⁴ Furthermore, this substrate can enhance angiogenic processes *in vitro*.¹⁰⁵ Roy *et al.*, for example, investigated the effects of platelet-rich fibrin matrix (PRFM) on EC behavior and showed that PRFM releases several growth factors, including VEGF-A and TGF- β , both of which are involved in endothelial remodeling and neovascularization.¹⁰⁶ Additionally, to tune the matrix’s mechanical properties closer to the stiffness of the organ *in vivo*, fibrin can be mixed with different ECM components and inorganic minerals; Ahn *et al.* developed a microfluidic model that encapsulates the 3D bone tumor microenvironment.¹⁰⁷ Since bone retains inorganic mineral hydroxyapatite (HA), which confers high stiffness and toughness properties, HA nanoparticles were added to the ECM to recapitulate the physiological microenvironment *in vitro*.

Pre-Patterned Vascularized MPS Platforms

Pre-patterned vasculature relies on preformed microfluidic channels to generate a lumenized vascular structure (Fig. 2B).^{108,109} Typically, several microfluidic channels are arrayed in parallel connected by a porous membrane that delineates different organ compartments (vascular, stromal, parenchymal, etc.). More complex vascular networks can be formed using sacrificial templates made of sugar or alginate.^{110,111} In most cases, to form a vessel-like structure these channels need to be coated with matrix to promote EC adhesion. This is accomplished by flushing the channels with relevant ECM proteins.

Laminin, Fibronectin and Collagen Coating—Options in EC coating techniques have been extensively described.^{112,113} Briefly, endothelial basement membrane proteins, such as laminin, collagen, or fibronectin are used to coat the surface and provide the native substrate necessary to achieve stable anchoring of ECs. Laminin, a cross shaped glycoprotein, is a major basement membrane component and a common EC *in vitro* substrate. Besides its structural role, laminins facilitate vascular tissue morphogenesis and homeostasis by regulating tissue architecture and adhesion-migration dynamics.¹¹⁴ Importantly, the use of laminin substrates is beneficial in vascularized MPS platforms that incorporate progenitor-like or iPSC-ECs, as it has been shown to promote commitment towards endothelial lineages.¹¹⁵ Fibronectin, another glycoprotein, is also a commonly used EC substrate coating.^{116,117} It benefits EC growth by promoting VEGF-induced differentiation¹¹⁸ and is efficiently absorbed onto PDMS,¹¹⁹ a common MPS biomaterial. Finally, collagen is a suitable ECM coating for EC attachment and broadly employed for biomedical engineering purposes.¹²⁰ As micropatterning studies uncover the extent to which cellular topographies regulate morphology, migration and mortality^{117,121} strategies to leverage collagen’s capacity to finely guide endothelial alignment have been employed. At increasing flow rates of injection, for example, collagen fibrils align parallel to experienced shear; ECs seeded on the aligned matrix migrate along the direction of fiber alignment, suggesting that the orientation of ECM regulates EC morphology.¹²² This technique for producing oriented collagen fibers can be applied to pre-patterned vascularized MPS system by controlling the device geometry and flow conditions of collagen necessary for effective EC attachment.

3D Bioprinted Vascularized MPS Platforms

3D bioprinted vascularized MPS platforms include those where tissues are generated layer-by-layer from a single-cell suspension (Fig. 2C).^{123–125} In recent years, 3D printing has emerged as an important biomedical tool. A key advantage of 3D printing is the ability to have precise control within a 3D space to generate complex geometrical features of vascular tissue *in vitro*. Cui *et al.*, for instance, utilized thermal inkjet printing technology to fabricate micro-sized vascular channels. Before printing, HMVECs were premixed with thrombin, which serves as the “bio-ink,” and printed onto a fibrinogen substrate. Thrombin then catalyzes fibrin gel polymerization, thereby minimizing structure deformation after printing. The authors found cells aligned themselves within the fibrin channel and proliferated to form a confluent lining.¹²⁶

As the base bio-ink material, an ideal hydrogel should show the following properties: printability, mechanical strength, and post printing gelation. Bio-ink printability depends on several parameters such as viscosity of the solution, surface tension of the bio-ink, and the ability to initiate crosslinking. If the bio-ink is highly viscous, for example, the 3D printed structures will have higher stability, but this requires higher extruding pressure during printing.¹²⁷ As individual printed filaments require enough strength and stiffness to maintain structural integrity after printing, a balance needs to be attained between printability and printed structure stability. Finally, hydrogel gelation is an important aspect in preserving the shape of bioprinted structures and minimizing structure collapse.¹²⁸ As 3D printing is a relatively young technology, the majority of research developed thus far focuses on fine tuning broader characteristics of bio-inks including printability, biocompatibility and ease of use; bio-ink specifics for optimal EC culture have yet to be developed.

The Promise of Tissue-Specific ECM in Next-Generation Vascularized MPS Platforms

Increasing evidence shows that tissue-specific ECM can promote site-appropriate differentiation of stem cells and maintain the appropriate phenotype *in vitro*.^{129,130} Sellaro *et al.*, for example, cultured sinusoidal ECs (SECs) on different substrates, including ECM derived from the liver (L-ECM), bladder (UBM-ECM), and small intestine submucosa (SIS-ECM)¹³¹ and showed that while SECs cultured on L-ECM maintain a differentiated phenotype for a minimum of three days, those cultured on UBM-ECM and SIS-ECM showed signs of dedifferentiation after 24 hours. These results verify tissue-specific ECM scaffolding optimally supports a unique population of cells *in vivo* and *in vitro* and is currently an underutilized within organotypic MPS platforms. Finally, tissue-specific ECM is proposed to have distinct tissue development roles through influencing cell differentiation, variation in pore diameter,^{132,133} the surface texture, and the rigidity and elasticity^{134,135} of the tissues.

As noted, most vascularized MPS platforms currently utilize standardized (non-tissue-specific) ECM coatings and hydrogels to promote endothelial attachment and maintain a vasculogenic potential. Only more recently have developers applied organ-specific ECM within MPS platforms harvested from decellularized tissues. Marturano-Kruik *et al.*, for example, incorporated ECs and bone marrow mesenchymal stem cells (MSCs) within a decellularized 3D bone matrix embedded microfluidic platform to create a perivascular

niche-on-a-chip.¹³⁶ The authors demonstrate bone marrow MSCs undergo phenotypical transition toward mural cell lineages and support the formation of capillary-like structures. Furthermore, ECM derived from diseased organs can also provide informative cues that support pathogenesis. Romero-López *et al.* compared the protein composition and stiffness of decellularized ECM from normal and colon tumor tissue.¹³⁷ After evaluating vascular network formation and tumor growth in each of the reconstituted matrices, the authors concluded that the tumor-derived matrix enhanced tumor cell proliferation and altered vascular network formation.

While decellularized ECM constitutes a perfect source of biological matrix possessing the organotypic molecular and mechanical properties necessary for tissue support, there are several obstacles to its universal application within MPS devices, such as limited tissue biopsy access, heterogeneity, and challenges associated with cell removal versus preservation of ECM composition.^{138,139} Thus, we believe that modifying the EC hydrogel scaffold or coating to mimic the important properties from the tissue *in vivo*, such as ECM composition, stiffness, and cell attachment is a more practical way to achieve tissue-specific ECM characteristics while current methods to purify organotypic ECM is currently inefficient.

Endothelial Cells under Flow Conditions Sense Shear

Under physiological conditions, the endothelium is exposed to various mechanical and hemodynamic forces due to interstitial and pulsatile blood flow. While radial forces caused by intravascular pressures are derived from the former, axial shear forces caused by friction are driven by the latter. Force and shear vary spatially and temporally throughout the vasculature depending on vessel diameter, wall thickness, geometry and pre- or post-capillaries. For instance, in straighter sections of blood vessels flow is laminar and largely unidirectional; at regions of blood vessel curvature, flow is more likely to be disturbed with stagnant dead volumes. Moreover, straighter vessels experience greater wall shear stresses than curved vasculature.^{140,141} Importantly, however, at the small diameters used in MPS systems the Reynolds number is low and the flow can safely be assumed to be linear. Existing in such a highly dynamic microenvironment, ECs integrate this collection of biomechanical cues through a suite of luminal transmembrane receptors, cell-cell junctions and cell-basement membrane interactions mediated by integrins. These signals modulate a variety of cellular responses including morphogenesis, cell function and gene expression.^{142,143} Increasingly, flow shear stress has been shown to regulate vascular network formation, remodeling, and stabilization.¹⁴⁴ Thus, in order to create a physiological environment for vessel formation and maintenance *in vitro*, precisely controlling flow dynamics within proper ranges is key to maintaining normal EC function within MPS devices. In this section we explore the interaction between flow shear and EC barrier junction and the regulation of EC homeostasis by shear.

Mechanotransduction on Endothelial Barrier Junctions

The most common mechanical force ECs experience derives from fluid flow. ECs utilize membrane bound mechanotransducers, such as G protein-coupled receptors, glyocalyx

and endothelial-specific junctional complexes to convert mechanical forces into biological responses in a process called mechanotransduction.¹⁴⁵ The Piezo1 ion channel, a newly discovered shear stress mechanosensor, senses pulsatile blood flow and triggers Ca²⁺ signaling, for example, which can result in the reorganization of ECs towards the polarity of the applied force.¹⁴⁶ Additionally, ECs maintain specialized cell-cell contacts via VE-cadherin-based adherens junctions to retain vascular integrity in a force-filled environment.¹⁴⁷

Effects of Flow Shear on Endothelial Phenotype and Homeostasis

Subjecting ECs to unidirectional or disturbed shear stress patterns has been shown to significantly alter cell morphology and phenotype.¹⁴⁸ To demonstrate this phenomenon, Song *et al.* designed a microfluidic model of angiogenic sprouting and studied the interplay of shear stress, transverse interstitial flow, and VEGF gradients in mediating sprouting morphogenesis.¹⁴⁹ In this model, liquid perfuses two adjacent channels lined with ECs sandwiching a collagen filled chamber that allows vessel sprouting. By controlling the flow configuration in the two-channel device, the direction and flow profile of interstitial flow across the collagen chamber could be determined, and VEGF gradients, which govern EC morphogenesis, could be established. In line with previous observations, they found that endothelial tip cells orientate against the direction of interstitial flow, and likely, the VEGF gradient.

In addition to modulating the morphology and migration of ECs, shear stress also regulates EC homeostasis. Several studies have reported that shear stress induced by blood flow triggers essential signaling pathways that regulate endothelial homeostasis, such as those leading to generation of reactive oxygen species (ROS) and nitric oxide (NO).^{150,151} To demonstrate this effect, Chen *et al.* targeted SIRT1, an important modulator in cardiovascular function in health and disease and found that overexpression of SIRT1 prevented endothelial senescence, whereas inhibition of SIRT1 caused premature senescence.¹⁵² The authors found that, compared to ECs cultured under static conditions, laminar flow at 12 dyn/cm² increased EC SIRT1 expression, thereby increasing NO bioavailability.

Flow Shear Regulates Vascular Morphogenesis

Vascular morphogenesis is a dynamic process regulated by shear stress. As the magnitude of flow changes, thereby impacting the levels of shear that ECs experience, vessels modify their diameter proportionally to restore the initial shear stress level.¹⁵³ This suggests that ECs maintain flow shear stress within a desired range. Baeyens *et al.* introduced the flow shear stress set-point theory, which stipulates that vessel deviation from a set point induces readjustment of vessel diameters.¹⁵⁴ More specifically, flow shear stress at the set point maintains vessel quiescence, while shear higher or lower than the set point triggers vessel remodeling. Thus, microfluidic platforms that can precisely regulate the flow dynamic field, including flow rate, shear, flow direction, and Péclet number (Pe) are a powerful tool to investigate the role of physiological mass transport during vasculogenesis. Hsu *et al.*,¹⁴⁴ for example, designed three microfluidic flow conditions: high interstitial flow (Pe>10) in the transverse and the longitudinal flow direction, an intermediate state that is close to normal

living tissue, and diffusion-dominant flow ($Pe < 0.1$) and reported that vasculogenesis can be induced under high interstitial flow and diffusion dominant flow, but not under intermediate state (normal) flow.

Controlling Dynamic Flow Fields in Microfluidic Platforms

MPS platforms benefit from microfluidic-based technology that enables the use of small culture medium volumes, control over chemical and physical cues in the microenvironment, and the ability to perform relatively high throughput experiments. Fluid flow can be driven in MPS platforms via several techniques. One of the simplest and most common methods utilizes hydrostatic pressure: two liquid reservoirs with different liquid heights generate passive flow from the higher reservoir to the lower. This simple method is used extensively as no external pumps are required. However, flow rate decay occurs over time as the pressure driven by the hydrostatic head diminishes.¹⁰⁰ As a result, regular supplement of liquid is needed to maintain liquid levels. To solve the issue of passive pumps, Wang *et al.* proposed a siphon-based micropump system with autofill function.¹⁵⁵ Alternatively, rocker-based platforms can be used to periodically reverse the direction of flow, thereby maintaining a reasonably uniform flow rate.^{156,157} Finally, active mechanical pumps, such as syringe pumps with fine tuning flow capabilities, enable stable flow rate over time.⁸³ Syringe pumps, however, are bulky and not suitable at scale.

The Vascular Niche and MPS Organ-Specific Identity

The vasculature was long described as a passive conduit for delivering oxygen and nutrients, aiding blood coagulation, and facilitating the transportation of immune cells. Only more recently has the vasculature been appreciated for its genotypic and functional diversity, including the unique microenvironment it generates, and its contributions to organ development, homeostasis and regeneration. Indeed, the milieu proximal to organotypic microvasculature (i.e. the vascular niche) harbors distinct endothelial extrinsic cues affecting cellular differentiation, survival and proliferation.^{158–160} These instructive interactions between the vasculature and its resident cells was first hinted at by the finding that stem cells often reside close, to and interact with, capillary beds.^{161,162} The influence of the vascular niche on stem cells, parenchymal cells and disease progression is becoming of increasing interest to investigators in multiple fields. In this section, we briefly describe the vascular niche, its role in organogenesis, homeostasis, repair, and disease progression, and how incorporating a vascular compartment can strengthen the physiological relevance of the next generation of MPS devices.

The Instructive Role of the Vascular Niche

While the transcriptional specification of the vascular plexus¹⁶³ and its anatomical development^{164,165} are reasonably well understood, the molecular basis of organotypic specialization is an emerging field. Our current understanding of organotypic vascular development largely relies on seminal studies of endothelial specialization in the liver,¹⁶⁶ pancreas,¹⁶⁷ and brain.¹⁶⁸ Capillaries permeate all developing tissues and maturation of organotypic endothelium occurs synergistically with its resident organ. In other words, the endothelium assumes a distinct genotypic profile induced by the parenchyma and in

return parenchymal differentiation is influenced by secreted, and likely transmembrane, endothelial-derived signals. It is not yet known whether this reciprocal developmental relationship extrapolates to all organs. While the molecular underpinnings of EC specialization remain unclear, the active influence of ECs on proximal tissue is better understood.

Organogenesis, Homeostasis and Repair—On a per organ basis, the vascular niche performs distinctive and versatile functions in response to local needs. The endothelium imparts an instructive influence on its microenvironment utilizing soluble (e.g. angiocrine factors)¹⁶⁹ and insoluble (e.g. ECM-cell and cell-cell) interactions. This influence begins during development and prior to the maturation of the circulatory system as the endothelium extensively orchestrates organogenesis and organ patterning.¹⁷⁰ In the case of several developing organs, including the liver¹⁶⁶ and pancreas,¹⁶⁷ embryological work demonstrates nascent vasculature is not only informative, but critical for organogenic processes. Following development, the best articulated function of the vascular niche is its homeostatic role in directing stem cell proliferation, self-renewal, and differentiation.^{171–173} The niche bathes stem cells in stoichiometrically relevant concentrations of angiocrine factors that control a quiescent versus pro-proliferative/differentiation phenotype. Moreover, EC-derived ECM also plays a key role in niche signaling as it not only contributes to the polarization of cells, influencing the symmetric and asymmetric cell division of stem cells, but also provides a basement membrane to plastic cells unable to deposit scaffolding proteins.^{174,175} Finally, the vascular niche is implicated in repair processes as a local modulator of regeneration, supporting the expansion of stem and progenitor cells¹⁷⁶ and controlling fibrosis.¹⁷⁷ Accordingly, the functional capacity of the vasculature is multifaceted and it can be seen as a broad gatekeeper of organogenic and homeostatic processes.

Disease Progression—An essential step in the pathophysiology of many diseases includes remodeling of the endothelium and maladaptation of the vascular niche. Many cancers, for instance, co-opt or recruit the vasculature.¹⁷⁸ This capacity not only allows the cancer to grow beyond its diffusion limit, but also provides access to a formative niche with the ability to modulate tumor progression, migration, and metastasis.^{169,179} Moreover, the cancer stem cell (CSC) hypothesis stipulates this rare population is responsible for the initiation, recurrence and therapeutic resistance of many malignant tumors.¹⁸⁰ In a parallel fashion to non-malignant stem cells, the vascular niche appears to fortify the apex cell of the tumor differentiation hierarchy and support neoplasms.¹⁸¹ It is thus unsurprising the vascular niche is heavily considered as a therapeutic target.^{182–184}

The Vascular Niche and MPS Platforms

Vascularized MPS Platforms Mimic Organogenesis—Organogenesis requires the integration of signals from several parallel inputs including chemical, mechanical, and cell-cell interactions, characteristics of which vascularized MPS models support and maintain. The proximity of the endothelium to developing tissue during organogenesis suggests that ECs may play an essential role during organ development and maturation. Arguably, the formation of on-chip microtissues utilizes several developmental pathways to reform a tissue from a single cell suspension. Incorporation of EC-guided morphogenic and maturation

processes can be utilized in tissue development *ex vivo* and do not require the use of organotypic¹⁸⁵ or even species-specific¹⁸⁶ endothelia. For example, when human embryonic stem cell-derived pancreatic progenitor cells are co-cultured with HUVECs or rat heart microvascular endothelial cells (rHMVECs), improved maturation was observed.¹⁸⁶ Thus, the regulatory function of the vasculature and its niche have the potential to induce marked improvements in the physiological capacity of all MPS platforms.

Stem Cell Culture and Vascularized MPS—While *in vivo* models have helped identify cells residing in vascular niches, manipulating 2D *in vitro* “microenvironments”, which have fewer degrees of freedom compared to 3D tissues, remains challenging if we want to study the contribution of niche components to surrounding tissues. Thus, vascularized MPS platforms recapitulating the 3D functional unit of organs are uniquely positioned to not only benefit from the instructive nature of the endothelium to improve the model’s fidelity, but also to elucidate mechanisms underlying spatial and temporal control of the vascular niche. Recently, a handful of vascular niche-related MPS platforms have been published, many of which demonstrate the capacity to maintain a stem cell population for an extensive time period, validate progenitor cells egression, and observe lineage maturation.^{101,136,187,188} The majority of current niche-focused MPS platforms recapitulate the bone marrow microenvironment, as the complex, multi-niche interactions amongst hematopoietic, osteoblast and vascular cell populations to facilitate hematopoiesis are almost impossible to create with 2D culture strategies. Glaser *et al.* created a bone marrow MPS platform that retains perivascular and endosteal niches in a dual chamber design.¹⁰¹ This MPS demonstrates the capability to maintain hematopoietic stem/progenitor cells for two weeks *in vitro* in either niche, track the differentiation of resident stem cells into the myeloid and erythroid lineages, and witness the intravasation of immature neutrophils into the adjacent microfluidic lines. Impressively, the platform incorporates an additional chamber used to study the trafficking of malignant cells into the adjacent perivascular and endosteal niches, providing insight into cancers that preferentially metastasize to different bone marrow niches. Thus, MPS platforms have the capacity to support multiple microenvironments and maintain proximal communication of the niches for the purposes of clinical and basic science research.

Conclusion

Although developments in MPS technology have emerged rapidly with several strategies to incorporate vessel conduits, including self-assembled, pre-patterned, and 3D bioprinted methodologies, barriers to translationally bridge *in vitro* platforms and clinical results remain. Ideally, a fully functional, organotypic vascular network, rather than a passive conduit, is needed to develop superior physiologically relevant MPS platforms. The vasculature has been proven to not only support nutrient delivery and metabolic processes, but also to play a vital role in organogenesis through unique molecular and phenotypic signatures. These complex functions need to be captured in MPS models so that the target tissue behaves *in vitro* as it does *in vivo*. This is equally true in patient-derived organoids where maintenance of *in vivo* structure and function is the goal. If vasculature is not incorporated into these tissues, they will rapidly lose important maintenance cues, and

as they grow will also become hypoxic, further shifting gene expression away from that present *in vivo*.¹⁰ The vascular niche also maintains stem cell populations. While a small handful of MPS platforms have successfully incorporated organotypic vasculature and its niche, the universal adoption of organ-specific vasculature within MPS platforms is unlikely to materialize soon, primarily due to sourcing issues. As a result, researchers must build MPS platforms that balance several engineering choices to get as close to an organotypic phenotype as possible.

In this review, we have discussed current developments in vascularized MPS platforms and future perspectives with regard to establishing organotypic models. EC sourcing is a critical consideration as it contributes to the tissues genotypic and phenotypic capacities and improves the fidelity of the vascularized MPS to the model organ. HUVECs, thus far, are the most common EC source as they are easy to manipulate and culture for experimental purposes. As iPSC technology matures, iPSC-derived ECs with the capacity to maintain organ and patient-specific properties are likely to become increasingly utilized to promote organotypic characteristics. Furthermore, a clearer understanding is developing of the crucial role ECM plays as an indispensable factor for organogenesis. ECM not only provides the mechanical support to maintain vascular organization, but also supplies the chemical cues necessary for EC homeostasis and tissue organogenesis. Besides utilizing the standardized hydrogels as ECM in MPS, decellularized 3D matrix from tissue *in vivo* and the modification of archetypal ECM will be required to promote functional tissue reconstruction, provide constructive cues, and generate a vascular niche. Flow-derived shear also plays a key role in vascular network formation and EC function in MPS platforms. Microfluidic technology is a powerful tool when designing MPS platforms since it can precisely tune the microenvironment, including flow dynamics and chemical gradients. By integrating considerations such as EC source, ECM and shear control into the MPS, the system will generate a unique microenvironment that benefits organ development and homeostasis. We envision that integration of biological substrates, microfluidic techniques and stem cell biology strategies will generate vascularized MPS platforms that more accurately model the complexities of specific tissues in health and disease.

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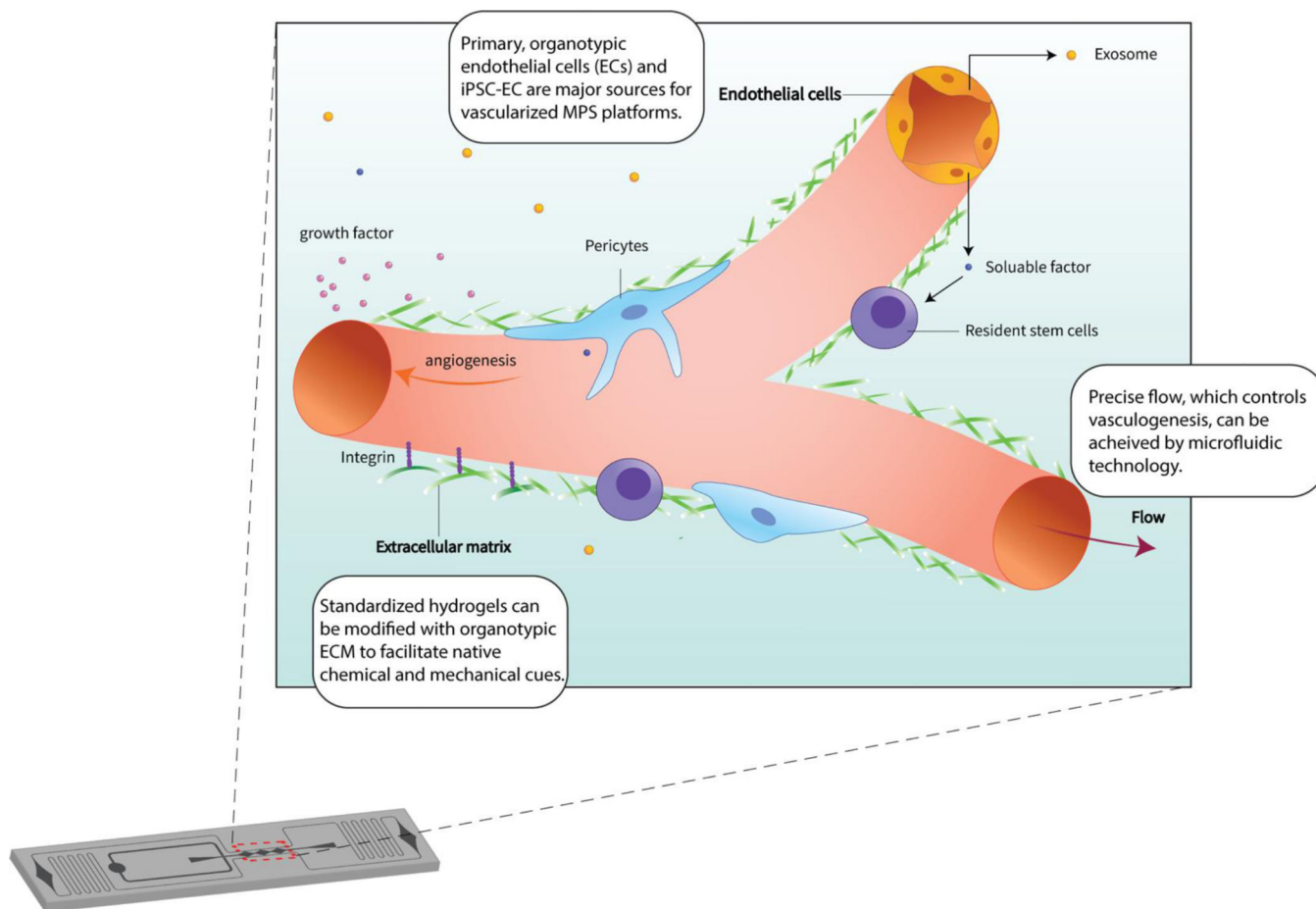


Fig 1. Core Engineering Strategies of Vascularized MPS.

Microfluidic technology precisely tunes the microenvironment, including flow dynamics and chemical gradients. By integrating considerations such as endothelial cell sourcing, extracellular matrix components and shear control into the MPS, the platform generates a unique *in vitro* microenvironment with the capacity to support a resident stem cell population.

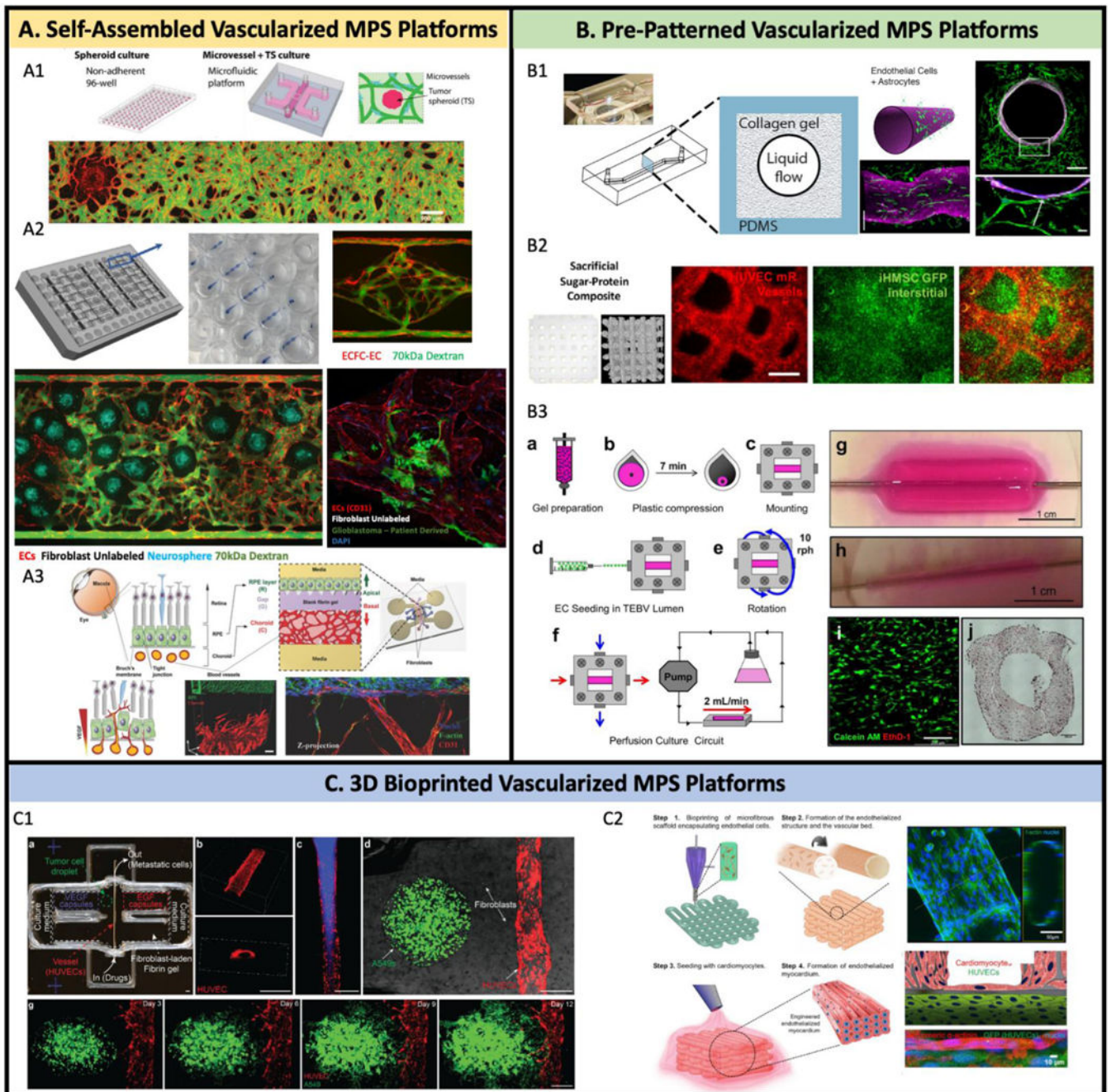


Fig 2. Strategies to build vascularized MPS.

(A) Self-assembled *in vitro* vascular network on MPS platforms. (A1) 3D vascularized tumor on-chip was built by co-culture 3D tumor spheroid, EC, and fibroblast into fibrin gel. (A2) 70kDa FITC dextran flowing through the upper “arteriole”, through the capillary network, and out through the low pressure “venule” at the bottom. Additional cell types can be seeded into the VMO as an *in vitro* model of other organs, vascular diseases, and cancer types and is amenable to the growth of neurospheres. Patient derived glioblastoma in the VMO tends to migrate towards and along the vasculature, suggesting the presence of a “preferred” vascular niche. (A3) A organotypic eye-on-a-chip model mimics the

pathogenesis of choroidal neovascularization (CNV) in the retinal pigment epithelium (RPE) choroid complex *in vitro*. **(B)** Pre-pattern vascularized MPS platforms. **(B1)** A 3D model of the human blood-brain barrier (BBB) within a cylindrical collagen lumen structure. **(B2)** Micron-resolution vascular features made by sugar-protein based sacrificial structure. **(B3)** Tissue engineered human blood vessels (TEBV) with human neonatal dermal fibroblasts (hNDFs) embedded in collagen gel. Coronary artery disease endothelial progenitor cells (EPCs) are seeded into TEBV lumen. **(C)** 3D bioprinted vascularized MPS platforms. **(C1)** Vascularized tumor models are built by 3D printing techniques to mimic cancer dissemination. **(C2)** Heart-on-chip with bioprinting endothelialized myocardium.

(A1) Adapted from ⁽¹⁰²⁾, with permission of John Wiley and Sons.
(A3) Adapted from ⁽¹⁹⁶⁾, with permission of John Wiley and Sons.
(B1) Adapted from ⁽¹²⁾, with permission of PLOS.
(B2) Adapted from ⁽¹¹¹⁾, with permission of Elsevier.
(B3) Adapted from ⁽²⁰⁷⁾.
(C1) Adapted from ⁽¹²⁵⁾, with permission of John Wiley and Sons.
(C2) Adapted from ⁽¹²³⁾, with permission of Elsevier.

Table 1.**Endothelial Cell Sourcing for Vascularized MPS Platforms**

<i>Primary ECs: Human Umbilical Vein Endothelial Cell (HUVEC)</i>					
Organ / Tissue	Vascularized MPS Study Focus	Vascular Network Formation Strategy	Lumenization?	Vascular barrier function assayed?	Ref.
Liver	Evaluation of anticancer bioactivity in the TME and hepatotoxicity in the liver	Pre-Patterned	No	-	[189]
Various Cancers	Endothelial regulation of chemotherapeutic transport	Self-Assembled	Yes	Yes	[102]
Ovarian Cancer	Platelet extravasation through the endothelium into tumor microenvironment	Pre-Patterned	Yes	Yes	[190]
Bone Marrow	Bone marrow pathophysiology	Pre-Patterned	Yes	No	[103]
Vasculature	Endothelial-to-mesenchymal transition axis	Self-Assembled	Yes	Yes	[13]
Various Cancers	Tumor heterogeneity and its influence on vasculature formation	Self-Assembled	Yes	No	[191]
Various Cancers	Tumor spheroid induced angiogenesis	Self-Assembled	No	-	[192]
Myocardia	Endothelialized-myocardium platform for cardiovascular toxicity evaluation	Bioprinted	Yes	No	[123]
Lung	Pulmonary toxicity of nanoparticles	Pre-Patterned	No	Yes	[193]
Lung	Anti-fibrotic drug nintedanib and its effect on vascular remodeling	Self-Assembled	Yes	Yes	[194]
Bone Marrow	Perivascular bone niche to study metastatic colonization of the bone	Self-Assembled	Yes	Yes	[136]
Lung	Pathophysiology of pulmonary thrombosis and advance drug development	Pre-Patterned	Yes	Yes	[195]
Retina	Outer blood-retinal barrier model	Self-Assembled	Yes	Yes	[196]
Glioma	Bioprinted glioblastoma tumors derived from patient-derived tumor cells	Bioprinted	No	-	[124]
Vasculature	Effects of ambient fine particulate matter on the vasculature	Self-Assembled	Yes	Yes	[197]
<i>Primary ECs: Organotypic Endothelial Cells</i>					
Kidney	Tubular–vascular exchange of solutes akin to native kidney tissue.	Pre-Patterned	Yes	Yes	[27]
Brain	Contributions of individual cell types of the blood brain barrier (BBB) to inflammatory stimuli	Pre-Patterned	Yes	Yes	[12]
Liver	Continuous zonated liver model for diseases modeling and ADME/TOX	Pre-Patterned	No	Yes	[198]
Kidney	Constructing a functional kidney glomerular-capillary-wall	Pre-Patterned	Yes	Yes	[199]
Vasculature	Endothelial barrier dysfunction associated inflammatory and hematological diseases	Pre-Patterned	Yes	Yes	[200]
Brain	Neurovascular microfluidic bioreactor for modeling of BBB function and testing of drug toxicity and permeability	Pre-Patterned	Yes	Yes	[201]
<i>Primary ECs: Endothelial Colony Forming Cell derived Endothelial Cells (ECFC-ECs)</i>					
Vasculature	Vascular inflammation and thrombosis	Pre-Patterned	Yes	Yes	[202]
Vasculature / Cancer	Reproducible vascularized micro-organs and tumors	Self-Assembled	Yes	Yes	[100,203]

Primary ECs: Human Umbilical Vein Endothelial Cell (HUVEC)					
Organ / Tissue	Vascularized MPS Study Focus	Vascular Network Formation Strategy	Lumenization?	Vascular barrier function assayed?	Ref.
Vasculature	Spatial and temporal control of oxygen tensions characteristic of <i>in vivo</i> biology	Self-Assembled	Yes	Yes	[204]
Vasculature	Large-scale perfusable microvascular networks	Self-Assembled	Yes	Yes	[205]
Bone Marrow	Hematopoietic stem/progenitor cell culture in parallel perivascular and endosteal niche	Self-Assembled	Yes	Yes	[101]
Colorectal Cancer	Capturing tumor heterogeneity, vascular disruption and TME interactions	Self-Assembled	Yes	Yes	[206]
Induced Pluripotent Stem Cells derived Endothelial Cells (iPSC-ECs)					
Vasculature	Developing a CDH5-mCherry reporter iPSC cell line amenable to form stable, perfusable microvessels	Self-Assembled	Yes	Yes	[52]
Progeria Syndrome	Elucidating diseased endothelium's role in Hutchinson-Gilford progeria syndrome	Pre-Patterned	Yes	No	[14]
Pancreas	Microvessel-pancreatic islet interactions	Self-Assembled	Yes	Yes	[51]