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Engineering in vitro vascular microsystems

Qiao Liu[1], Guoliang Ying[1,2,3], Chenyan Hu[1], Lingyu Du[1], Huaiyi Zhang[1], Zhenye Wang[1], Hongyan Yue[1], Ali K. Yetisen[4], Guixue Wang[2], Yang Shen[1,2,3]✉ and Nan Jiang[1,2,3]✉

Abstract

Blood vessels are hierarchical microchannels that transport nutrients and oxygen to different tissues and organs, while also eliminating metabolic waste from the body. Disorders of the vascular system impact both physiological and pathological processes. Conventional animal vascular models are complex, high-cost, time-consuming, and low-validity, which have limited the exploration of effective in vitro vascular microsystems. The morphologies of micro-scaled tubular structures and physiological properties of vascular tissues, including mechanical strength, thrombogenicity, and immunogenicity, can be mimicked in vitro by engineering strategies. This review highlights the state-of-the-art and advanced engineering strategies for in vitro vascular microsystems, covering the domains related to rational designs, manufacturing approaches, supporting materials, and organ-specific cell types. A broad range of biomedical applications of in vitro vascular microsystems are also summarized, including the recent advances in engineered vascularized tissues and organs for physiological and pathological study, drug screening, and personalized medicine. Moreover, the commercialization of in vitro vascular microsystems, the feasibility and limitations of current strategies and commercially available products, as well as perspectives on future directions for exploration, are elaborated. The in vitro modeling of vascular microsystems will facilitate rapid, robust, and efficient analysis in tissue engineering and broader regenerative medicine towards the development of personalized treatment approaches.

Introduction

The synchronized functioning of the human body relies on the efficient transportation of blood through vascular networks, which is essential for maintaining cellular function, absorbing necessary nutrients, and regulating cell homeostasis. According to the World Health Organization's top ten causes of death, vascular diseases have always been listed as one of the main causes of death worldwide. The number of deaths from cardiovascular diseases increased from 12.1 million in the 1990s to 18.6 million in 2019, while the incidence of cardiovascular diseases almost doubled, from 271 million in 1990 to 523 million in 2019, imposing a significant burden on human health[1]. Despite the remarkable advances in treatment and prevention, many physiological and pathological mechanisms underlying vascular diseases remain undefined. Hence, it is imperative to gain a comprehensive understanding of blood vessels and accelerate the development of therapeutic approaches. Over the past few decades, in vivo animal models and conventional in vitro two-dimensional (2D) cell culture models for vascular research have served as the gold standards[2]. Although animal models can replicate the complicated in vivo environment, there exist significant genetic differences between animals and humans. The development of an animal model typically requires several weeks and a costly special diet, while successful modeling always necessitates the sacrifice of many animals, resulting in an economic burden and time consumption. Additional testing equipment is needed to characterize the disease progression in vivo models, which further increases the financial burden. Additionally, the extensive use of experimental animals raises ethical concerns[3]. Conventional 2D cell culture can partially replace and reduce the use of laboratory animals, as well as employ human-derived cells

Correspondence: Yang Shen (shenyang@scu.edu.cn) or Nan Jiang (jiangnansophia@scu.edu.cn)
[1]West China School of Basic Medical Sciences & Forensic Medicine, Sichuan University, Chengdu, China
[2]Jinfeng Laboratory, Chongqing, China
Full list of author information is available at the end of the article
These authors contributed equally: Qiao Liu, Guoliang Ying

to decrease species diversity. However, it faces challenges to replicate a complex three-dimensional (3D) environment in vitro, including limited complexity, short lifespan, difficulties in replicating human immunoregulation, and the need for rigorous validation to ensure they accurately mimic human disease processes[4]. As a result, it fails to adequately mimic human physiological and pathological processes. These issues have impacted the precision of scientific deductions, prompting the exploration of in vitro models of microsystems.

The emergence of in vitro 3D vascular modeling has presented new solutions to these issues: (i) reducing the reliance on a large number of experimental animals, aligning with the internationally respected 3 R animal protection principle (reduction, refinement, and replacement); (ii) providing simulated microphysiological systems that enable co-culturing various cell types within a specific space, while also allowing for adjustable perfusion conditions and mechanical forces necessary for cell growth and organ formation; and (iii) incorporating sensors and response elements that allow for localized detection of molecules, providing convenient and rich data[5]. In vitro 3D vascular microsystems have demonstrated higher physiological correlation compared to conventional models, enabling accurate and reliable conclusions to be drawn and explaining numerous abnormal phenomena observed through historical experimental data.

Significant advancements have been made in modeling in vitro vascular platforms over the last decade[6]. Typical elements of an in vitro vascular microsystem include: (i) culturing living cells, such as monolayer vascular endothelial cells (VECs), vascular smooth muscle cells (VSMCs), and other pericytes, which are correctly arranged to form an integrated vascular structure with barrier function. Additionally, a supporting structure is necessary to provide the required extracellular environment for cell growth and development; (ii) accurately replicate the hemodynamic conditions in vivo, and (iii) examine cell response related to signaling. The vascular cells in in vitro microsystems can respond appropriately to in vitro stimuli, and these responses are easily measurable. However, due to the complexity of internal and external factors affecting the occurrence and development of vascular disease, it is challenging to fully replicate complex vessels in vitro. By extracting specific essential factors and variables, a simplified model of blood vessels can be established to investigate biological phenomena and processes. The design concept for in vitro vascular microsystems should aim to standardize and simplify while maintaining physiological relevance.

Replicating complex in vivo vascular structures and microenvironments with high fidelity would represent a significant milestone. As modeling in vitro vascularized microphysiological systems continues to advance, the simulations become more similar to in vivo microenvironments. This includes transitioning from simple, straight blood vessels to complex geometries, moving from single-cell cultures to co-cultures of various cell types on a chip, shifting from static culture to dynamic ones, adopting additive manufacturing technology instead of conventional techniques, and replacing microscope observation with real-time monitoring. Due to the intricate nature of the internal environment, it is imperative that in vitro vascular microsystems consider all aspects of in vivo models to replace animal experiments. This review presents strategies for constructing in vitro vascular microsystems by examining manufacturing methods, selecting supporting materials, arranging seeded cells, discussing their applications, and providing future strategies.

Structures and properties of blood vessels

The blood vessel network extends throughout the human body like an intricate web, efficiently transporting life-sustaining oxygen and essential nutrients to every tissue and organ, while simultaneously eliminating metabolites[7]. Blood vessels exhibit unique structural features that are essential for their physiological functions. A comprehensive understanding of these features is critical for engineering in vitro vascular microsystems.

Vascular structure

Blood vessels exhibit unique structural features that are essential for their physiological functions. A comprehensive understanding of these features is critical for engineering in vitro vascular microsystems (Fig. 1a). The innermost layer of blood vessels, known as the tunica intima, consist of a monolayer of VECs. Uniformly aligned VECs serve as a critical barrier between blood flow and surrounding tissues, regulating the exchange of oxygen, nutrients, and metabolic waste. VECs also control vascular tone and maintain homeostasis by producing cytokines in response to blood flow dynamics[8]. The tunica media, primarily composed of VSMCs and elastic fibers, is responsible for regulating vascular tone through contraction and relaxation[9,10]. The tunica adventitia, composed of fibroblasts and connective tissue, provides structural support to blood vessels and plays a key role in producing reactive oxygen species and vasoactive hormones[11].

Vascular hierarchies

The vascular network spans a wide range of vessel sizes and geometries. In addition to reconstructing the cell hierarchy of the vessel, it is also necessary to consider the hierarchies of type, diameter, and geometry. The vasculature in vivo is composed of arteries, veins, and

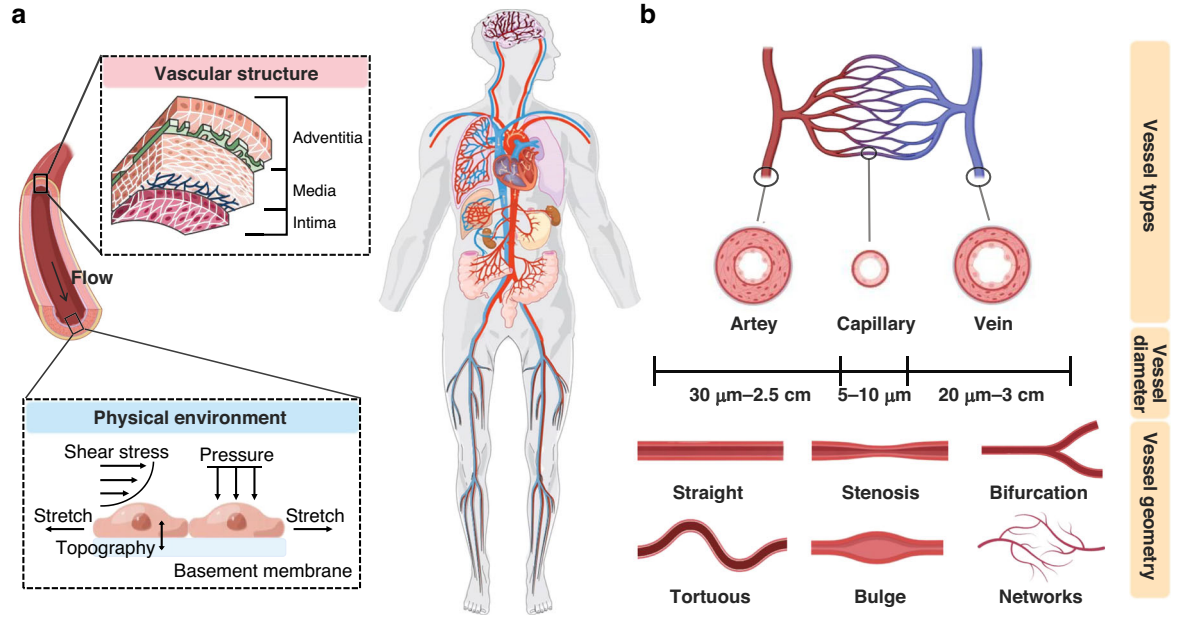

Fig. 1 Schematic representation of the structure, physical environment, and types of human blood vessels. a Blood vessels share a general three-layer feature, including the intima, media, and adventitia. They are generally affected by several mechanical forces, including shear stress acting on VECs and pressure from blood flow, stretch from vessels, and topography from the basement membrane. **b** The types of blood vessels can be divided into arteries, capillaries, and veins, according to the differences in their size, structure, and functions. Based on the complex morphology of blood vessels, multiple geometry types are identified, such as stenosis, bifurcation, and tortuous

capillaries. Arteries and veins, with diameters ranging from tens of micrometers to several centimeters, feature multi-layered walls that are designed to withstand varying hemodynamic pressures[12,13]. In contrast, capillaries are much smaller, typically ranging from 5 μm to 10 μm in diameter, and consist of a single layer of endothelial cells, enabling efficient nutrient and waste exchange (Fig. 1b)[7]. The technical requirements for modeling vascular hierarchies involve a combination of multi-scale fabrication, geometric fidelity, and pressure compatibility. For multi-scale fabrication, microfluidic systems such as soft lithography are appropriate for creating capillaries (<20 μm), whereas sacrificial molding is more suitable for constructing macrovessels (>1 mm)[14]. Maintaining geometric fidelity is critical, particularly in designs featuring stenosis or bifurcation, which require high-resolution 3D bioprinting techniques[15]. Additionally, to replicate physiological conditions, macrovessel models must incorporate pulsatile flow systems, such as syringe pumps with integrated pressure sensors, to mimic arterial pressures ranging from 80 to 120 mmHg[16].

Physical environments

The physical environment within blood vessels is highly complex, subjecting vascular cells to a variety of mechanical forces, primarily hemodynamic forces, such as shear stress, cyclic stretch, and circumferential pressure. Additionally, vessel forces, including the mechanical and topographical properties of the basement membrane, as well as intravascular forces, such as blood viscoelasticity and imagination, also play significant roles (Fig. 1a)[17]. These forces exert an influence on vascular cell behavior, particularly in VECS and VSMCs, which can sense mechanical stimuli and transduce them into biochemical signals[18]. For instance, high shear stress promotes an anti-inflammatory and antithrombotic phenotype in VECs, while turbulent flow induces pro-inflammatory and pro-coagulant effects[19]. VSMCs in the medial layers primarily experience cyclic stretch due to the pulsatile pressure exerted by the heart[20]. A relatively stable physical environment is crucial for maintaining the integrity of vascular structure and function, as well as promoting healthy vasculature development. This involves the dynamical application of fluid flow to simulate shear stress, the mechanical stretching of vessel models to replicate pulsatile pressure, and the precise imitation of blood viscosity.

Overall, it is crucial to establish the physiological structure of blood vessels when reconstructing vascularized microphysiological systems. To accurately model blood vessels in vitro, several technical aspects should be considered: (i) Cellular co-culture. A combination of VECs, VSMCs, and extracellular matrix (ECM) components is required to mimic the native vascular structure,

hierarchies, and dynamic behavior. (ii) Hemodynamic simulation. Blood flow should be simulated through perfusion systems to recreate shear stress and cyclic stretch, essential for cellular behavior. (iii) Supporting materials. Supporting materials are necessary to be biocompatible and can support cell growth, as well as maintain structural integrity under mechanical forces. (vi) Microscale fabrication. Advanced manufacturing methods, such as 3D printing and microfluidics, are employed to design precise vascular geometries, including branching and curvature, which affect flow dynamics and cell responses.

Manufacturing methods for in vitro vascular microsystems

The critical step in constructing in vitro vascular microsystems is the endothelialization of VECs, which leads to the formation of the barrier function of blood vessels. Approaches to in vitro endothelialization can be primarily categorized as predesigned patterning and self-assembly. The predesigned patterning approach involves predefining the structure, scale, and geometry of blood vessels. To name a few, the desired channels and networks can be achieved by driving and restricting vascular cells, eliminating the need for self-organized tubulogenesis and anastomosis through preconstructed microchannels[21]. As well, it is easier to regulate mechanical stimuli (fluid shear stress, cyclic stretch, circumferential pressure, and ECM stiffness) and biochemical stimuli (blood cells, growth factors, inflammatory factors, and drugs).

The self-assembly approach is more similar to the growth and development of in vivo vascularization, compared with predesigned patterning, which undergoes morphogenesis and self-assembly into vascular networks. Studies have demonstrated the potential of VECs to self-assemble into functional blood vessels, which are formed and stabilized through their association with VSMCs or pericytes[22]. VECs are encapsulated in hydrogels and directly seeded within the device in vitro. By adding growth factors, these VECs can self-organize, resulting in the formation of stable and perfusable 3D vascular networks. Vascular self-assembly can also occur in mature tissues during pathological conditions such as tumor development, and this method is commonly utilized to screen antiangiogenic drugs for cancer treatment[23,24].

The combination of predesigned patterning and self-assembly, may be a more effective approach to constructing physiologically relevant models with vascularization[25,26]. This section will focus on the advanced manufacturing technologies that enable the creation of these in vitro vascular devices, highlighting how precise engineering and natural self-organization can be integrated to meet research needs.

Advanced templating methods

Templating methods facilitate the creation of vascular networks with pre-defined geometries, addressing the challenge of replicating in vivo-like lumen structures. Upon the properties of the support matrix, it can be classified into hard templates and soft templates. Hard templating methods employ high-stiffness templates such as needles, optical fibers, rods, and metallic wires to support matrix materials[27,28]. These methods are straightforward, cost-efficient, and widely utilized for constructing in vitro vascular microsystems, providing control over size, structure, and configuration[29,30]. Nevertheless, conventional templating methods frequently encounter difficulties in replicating highly curved, branched, or 3D vessel networks that are necessary for more physiologically relevant models. The soft-templating technique presents distinct advantages in overcoming these challenges while balancing complexity, speed, and reproducibility.

Direct 3D-printed templating

Direct 3D printing overcomes the challenge of replicating complex and highly curved vascular geometries that are difficult to achieve using traditional molding techniques. Advances in 3D printing techniques now enable researchers to fabricate vascular structures featuring intricate curvatures and detailed branching patterns. For example, stereolithography (SLA) has been used to generate models of healthy and diseased coronary arteries, incorporating geometries such as stenotic regions that mimic real-world vascular occlusions. This approach allows for the investigation of how vessel geometry impacts blood flow dynamics, platelet aggregation, and clot formation under physiological conditions (Fig. 2a)[31]. These models provide critical insights into cardiovascular diseases, where vascular geometry plays a crucial role in disease progression. Moreover, direct 3D printing offers a high degree of precision in scaling the size of vascular channels and networks to create models that are more representative of the human vascular system[32]. This technique is particularly beneficial for replicating the full range of vascular structures, from larger arteries to capillary networks, which have been a significant challenge in earlier microfluidic models.

Sacrificial templating

Sacrificial templating provides a solution to the challenge of manufacturing complex vascular networks with elaborate geometries while ensuring functional perfusion. In dissolvable-based sacrificial molding, a negative mold is created using a dissolvable template, which is then enclosed within pre-polymer solutions of hydrogels or polydimethylsiloxane (PDMS). After polymerization, sacrificial materials are dissolved or melted and flushed

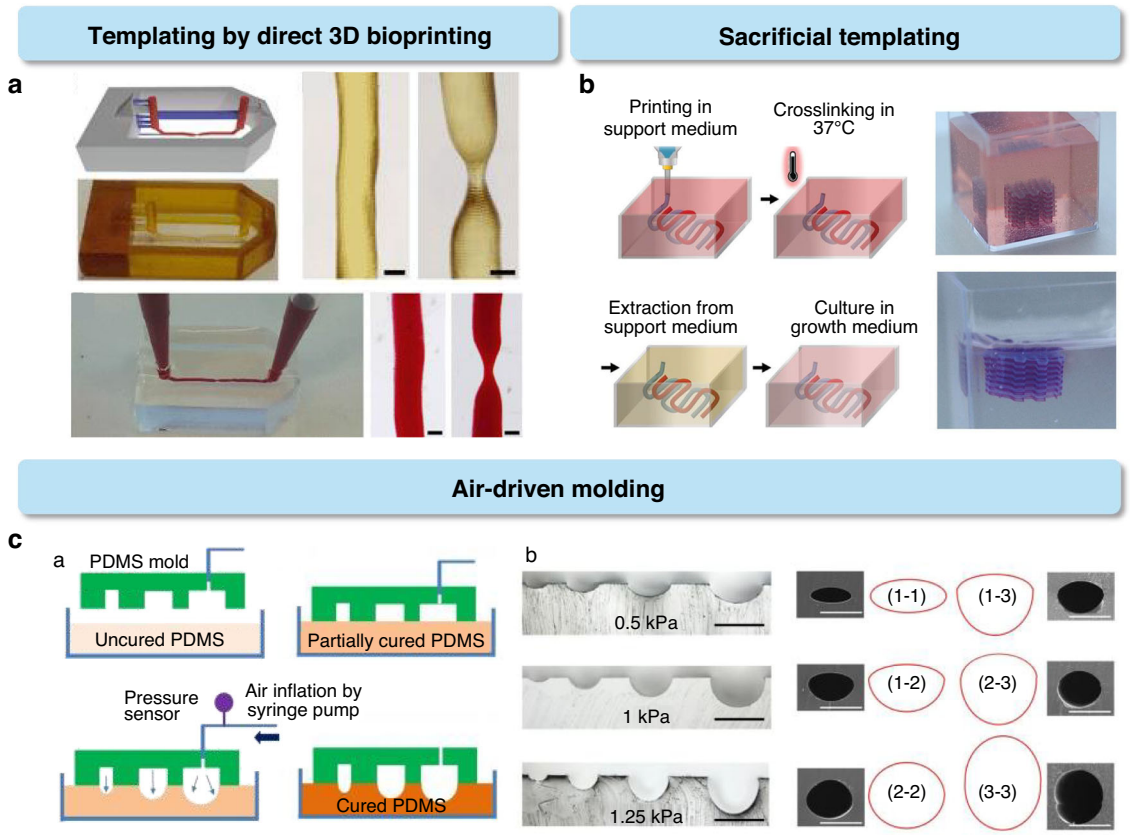

Fig. 2 Advanced templating methods for in vitro vascular microsystems. a 3D microfluidic models of a healthy and a stenotic vessel fabricated by direct 3D bioprinting. Scale bars: 370 μm[31]. Copyright 2017, Royal Society of Chemistry. **b** Steps of printing personalized structures in supporting media using sacrificial ink[35]. Copyright 2019, Wiley-VCH. **c** Air-driven molding strategy: a Schematic of fabrication steps of semi-circular microchannels. b Brightfield images of the semi-circular channels at different pressures and combining different semi-circular channels to form circular channels. (1) is defined as the condition for under deformation, (2) is the semi-circular, and (3) is the over deformation. Various cross-sectional channels of 500 μm in width can be obtained by combining the different fabrication conditions of the first half and the second half of the channel. Scale bar: 500 μm[41]. Copyright 2020, Elsevier

out, leaving behind intricate microchannels and networks[33].

The main challenge that sacrificial templating addresses is the ability to create vascular microsystems with high geometric complexity, such as multi-tiered vascular networks and branching structures, which are essential for accurate modeling microcirculation[34]. A recent tendency involves embedding 3D-printed sacrificial structures into hydrogel matrices. Using personalized bioinks, a layer-by-layer approach was developed to print blood vessels within cardiac tissue. Perfusable patches with triaxial lumens were fabricated and extracted via enzymatic or chemical degradation (Fig. 2b)[35]. Embedding thermo-responsive materials has improved the ease of removing sacrificial structures and rapid functional endothelialization, providing more flexible designs[36]. Despite these advancements, a major challenge remains optimizing the sacrificial material removal process to minimize damage to the microchannels and maintain cell viability during the fabrication process[37,38].

Air-driven molding

Air-driven molding employs a gas stream to displace a viscous pre-polymer solution, thereby forming microchannels with controlled geometries. The gas stream facilitates easy manipulation of the channel diameter and shape during polymerization, making it an efficient and cost-effective approach for simple vascular structures. It minimizes mechanical stress on delicate materials and enables the precise creation of circular vascular lumens, ensuring smooth and uniform structures that are essential for accurate blood flow simulations and realistic vascular microsystems[39,40]. For example, Nguyen et al. utilized inflated gas pressure to manipulate partially cured PDMS using simple bench-top equipment, resulting in the fabrication of circular channels with varying diameters and stenosis vessels with different geometries (Fig. 2c)[41]. While air-driven molding is effective for generating microchannels with variable diameters, it has limitations when it comes to fabricating more intricate vascular networks with complex branching or curved geometries.

Micromolding

Physical molding and templated microstructures by manipulating elastomers and thermoplastics have been developed to construct vascular microchannels. This manufacturing process is more flexible than templating methods, which can easily produce microchannels with features smaller than 100 μm.

Micromilling

Micromilling is a process that utilizes cutting tools to remove bulk materials and create microscale features. Computer numerical control (CNC) micromilling, used mainly in plastics, enables the direct creation of parts or molds for subsequent fabrication steps like injection molding and embossing. This method is particularly beneficial for the rapid prototyping of vascular structures without the requirement for molds or masks. Micromilling addresses the challenge of promptly manufacturing vascular models with precise features, allowing for the creation of small-scale channels that mimic microvascular networks. Nevertheless, the precision of micromilling is restricted by the accuracy of the cutting tools, and surface roughness in the microchannels can impact cell behavior, such as adhesion and migration[42]. Despite these limitations, micromilling is a valuable tool for rapid prototyping and testing simple vascular models, enabling efficient model development for basic studies and early-stage experiments (Fig. 3a)[43].

Soft lithography

Since soft lithography was first introduced by the Whitesides group, it has been widely utilized in the fields of medicine and pharmaceuticals, due to its ability to produce PDMS devices with biocompatibility, throughput, and transparency for imaging[44,45]. Using hard masters as molds, soft lithography generates elastic microstructures or micromolds through traditional processing techniques, such as micromilling, lithography, laser, etc. Standard soft lithographic processing involves using a photomask to create patterns on a substrate, which are then transferred to a PDMS mold for further treatment[46]. Soft lithography is especially valuable for creating vascular models featuring detailed and reproducible characteristics, offering a solution to the challenge of fabricating high-resolution and perfusable vascular networks (Fig. 3b)[47]. The primary challenge addressed by soft lithography is the capacity to produce high-quality vascular models with precise geometries, suitable for studying cellular behavior in response to mechanical and biochemical stimuli. However, the method can be time-consuming and requires a cleanroom environment, making it less ideal for rapid prototyping or large-scale fabrication.

As a consequence, numerous researchers have integrated micromolding techniques with soft lithography to fabricate hard masters and soft microsystems in order to obtain the final structure. By combining micromilling with soft lithography, the non-photolithographic strategy for constructing multi-tiered and cylindrical microstructures was developed[47]. Laser technologies can be used to create micrometric structures, mimic atheroma plaque stenosis, and design personalized blood vessels with specific geometries[48]. To overcome the limitations of PDMS and take advantage of the benefits of polystyrene (PS), while achieving the goals of rapid prototyping, microfabrication using soft lithography techniques can be applied to PS.[49,50]. Micromolding typically produces rectangular structures, and this limitation can be addressed by using spin-coating technology to achieve circular vascular shapes[51]. In addition, although micromolding can produce uniform semi-closed channels, creating closed channels requires tedious alignment and bonding.

Laser ablation

Laser ablation offers a solution to the challenge of rapidly producing high-quality and intricate vascular models with fine resolution and minimal material waste. Laser ablation allows for the direct generation of vascular networks without the requirement of molds, making it a cost-effective and efficient alternative for fabricating vascular models within a short period. The microchannel is patterned onto the PDMS thin film using the front-surface scanning method in laser ablation, which includes continuous photothermal pyrolysis guided by a continuous-wave laser from above. By controlling the laser intensity, the size of the structures can be accurately modified (Fig. 3c)[52].

Recently, Shin et al. developed monolithic quasi-3D digital patterning of PDMS using successive laser pyrolysis, taking advantage of the self-sustaining photothermal effect of a continuous-wave laser to improve patterning quality. This method exploited the pyrolysis property of PDMS to produce opaque material with enhanced laser absorption, enabling efficient photothermal energy generation. With the removal of by-product, 2D or 3D microstructures can be rapidly patterned without post-processing[53]. A separate study demonstrated superior laser ablation efficiency on poly(glycerol sebacate) and poly(APS), creating microfluidic systems mimicking vasculature[54]. Laser ablation has been proven to be highly effective for rapid prototyping and generating simple vascular models, but more complex geometries might require additional techniques to achieve the desired level of detail.

Direct 3D bioprinting of vessel-like models

Whether using template-based methods or microfabrication, one of the limitations resides in the

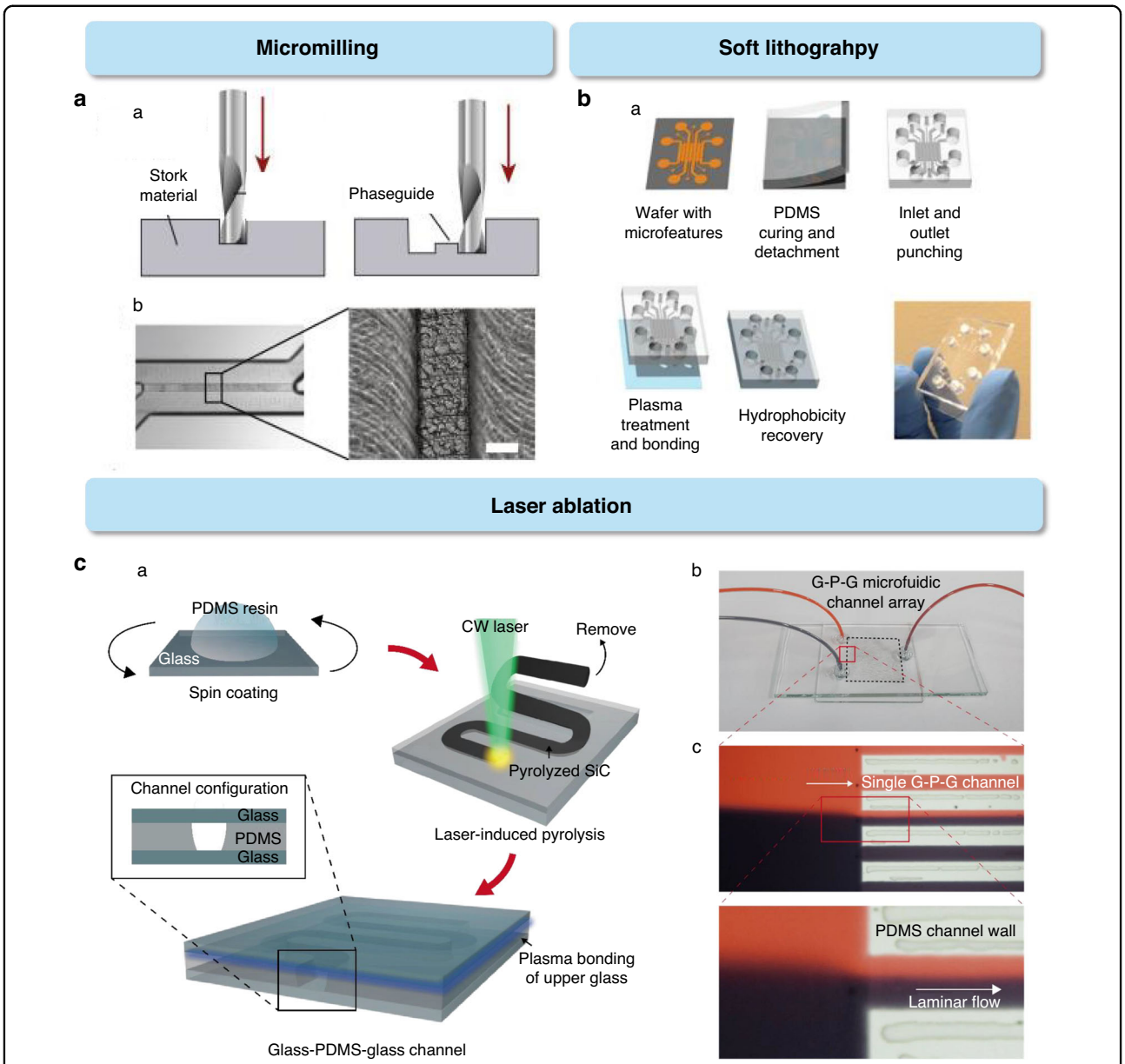

Fig. 3 Micromolding for in vitro vascular microsystems. a a Schematic of phaseguide and microchannel milling. b Phase contrast image of micromilled phaseguide and microchannels. Scale bar: 50 μm[43]. Copyright 2020, Elsevier. **b** A PDMS device was made with standard soft lithography techniques[315]. Copyright 2017, Springer. **c** a Concept illustration of the process of laser ablation. b Images of the device with inlet and outlet tubes, c optical microscopy images of multiple channels showing laminar flow developed[52]. Copyright 2021, MDPI

requirement for constructing intricate structures of tissues and organs. Direct 3D bioprinting is an innovative approach that directly tackles the challenge of creating intricate vascular networks using bioinks. This method enables the precise positioning of cells, scaffolds, and growth factors to form vascular structures that closely resemble the in vivo microenvironment. The ability to print living cells directly into the vascular microsystems is a breakthrough in the field, allowing for the creation of functional blood vessels that can respond to physiological stimuli[55,56]. Nozzle-based and optical-based methods are two broad categories of direct 3D bioprinting.

Nozzle-based bioprinting

Nozzle-based bioprinting (NBB) has emerged as an established technology in the creation of vascular networks due to its capacity to precisely place cells, growth factors, and scaffolds. The primary challenge in vascular microsystems engineering addressed by NBB lies in the requirement for high-resolution control over the

placement of multiple cell types in a predictable manner to form functional vascular networks. The bioink is propelled through nozzles by applying force fields, allowing for controlled extrusion[57]. Although NBB is cost-effective and widely available, it also faces challenges such as the high shear stress exerted on cells during extrusion, which adversely impacts cell viability and limits its application in some tissue-engineering contexts[58]. Nevertheless, NBB remains a reliable method for fabricating multi-scale cell-laden vascular constructs. Notably, droplet-based bioprinting (DBB) and extrusion-based bioprinting (EBB), two primary techniques within NBB, are frequently utilized:

DBB DBB operates in a manner similar to inkjet printing, where bioink droplets replace traditional ink and are deposited in precise locations on a substrate to build 2D cell patterns that eventually form 3D structures[59,60]. The primary breakthrough that DBB provides in the context of vascular microsystem modeling is its ability to print high-resolution structures (~50 μm) at high speeds (up to 10,000 droplets per second)[61]. This enables the creation of smaller, functional blood vessels with precise control. For instance, Kang et al. developed biomimetic 3D alveolar barrier models by using drop-on-demand inkjet printing, enabling high-resolution cell deposition. This model replicated the structural complexity of alveolar tissue (10 μm) and accurately mimicked tissue-level responses to influenza, presenting potential as a viral infection model (Fig. 4a)[62]. The application of DBB technique to complex biomimetic models, such as functional skin and cartilage tissue, demonstrates its significant potential in replicating the complexity of human tissues[63,64]. However, DBB faces the limitation of printing highly viscous bioinks due to the potential nozzle clogging, a challenge that has been partially overcome by optimizing ink properties and printer designs[65].

EBB Similar to the fused deposition modeling of conventional 3D printing, EBB utilizes pneumatic or mechanical extrusion systems for continuous filament extrusion of cell-laden bioinks from needles. The pre-defined structures are formed after crosslinking and solidification[66]. One of the most significant technical advancements within EBB is the development of coaxial bioprinting, which allows the deposition of multiple flow streams in concentric rings through a single nozzle, enabling the fabrication of intricate vascular microchannels and facilitating cellular co-culture[67]. This breakthrough is particularly valuable for the fabrication of intricate vascular 3D structures and functional tissue and organ constructs featuring in vitro vascular-like microchannels. A triple-layered arterial construct with tunable shapes and dimensions was developed utilizing in-bath coaxial cell-printing technology. This model has the potential to generate vascular tissues that can respond to different stimuli. Under turbulent flows, multiple vascular tissues enabled the replication of the key early atherosclerosis (AS) events[68]. Furthermore, recent innovations such as sequential printing in reversible ink templates (SPIRIT) are overcoming the limitations of traditional embedded 3D bioprinting by incorporating complex tissue structures and perfusable blood vessels (Fig. 4b)[69].

Light-based bioprinting (LBB)

The resolution of the bioprinter imposes limitations on the size of manufactured in vitro vascular microsystems. LBB employs laser energy to precisely control the polymerization of photosensitive bioinks, offering significant advantages over nozzle-based methods, particularly in terms of printing speed, resolution, and cell viability[70]. Importantly, LBB also overcomes the issues of nozzle interference and blockage, which are critical challenges in the engineering of 3D bioprinting vascular microsystems. The growing application of LBB in the creation of in vitro tissue and organ models represents a major technological advancement. Here, we focus on introducing two techniques—photopolymerization-based bioprinting (PBB) and direct laser patterning, which are commonly used for the construction of in vitro vascular microsystems.

PBB PBB is broadly categorized into SLA and digital light processing (DLP). SLA, a technique that originated in the 1980s, employs light-induced curing of a pre-polymer solution layer-by-layer to fabricate 3D structures[71]. While SLA offers high resolution, its relatively slower print speed, resulting from point-by-point curing, can be a constraint. However, advancements such as projection-based SLA and DLP have addressed this issue, allowing for faster and more efficient printing. In DLP-SLA, a liquid crystal display or a digital micromirror device is used to project designed patterns onto 2D slices for layer-by-layer photopolymerization. It enables the rapid creation of complex geometries with high resolution and speed[72]. DLP bioprinting, in particular, is considered a next-generation microfabrication technique for creating intricate vascular structures. A notable breakthrough in the use of DLP for vascular modeling was achieved by Ming Yang et al., who employed multi-material DLP bioprinting to fabricate multi-component, cell-laden hydrogel constructs, including bone, liver lobules, and vascular networks. Particularly, they replicated the mechanical moduli of arteries, veins, and capillaries, each designed to mimic the distinct mechanical properties of vascular components (Fig. 4c)[73]. The utilization of a container filled with photocurable bioink in DLP bioprinting enhances the stability of printed structures, and eliminates the challenges of nozzle clogging or shear

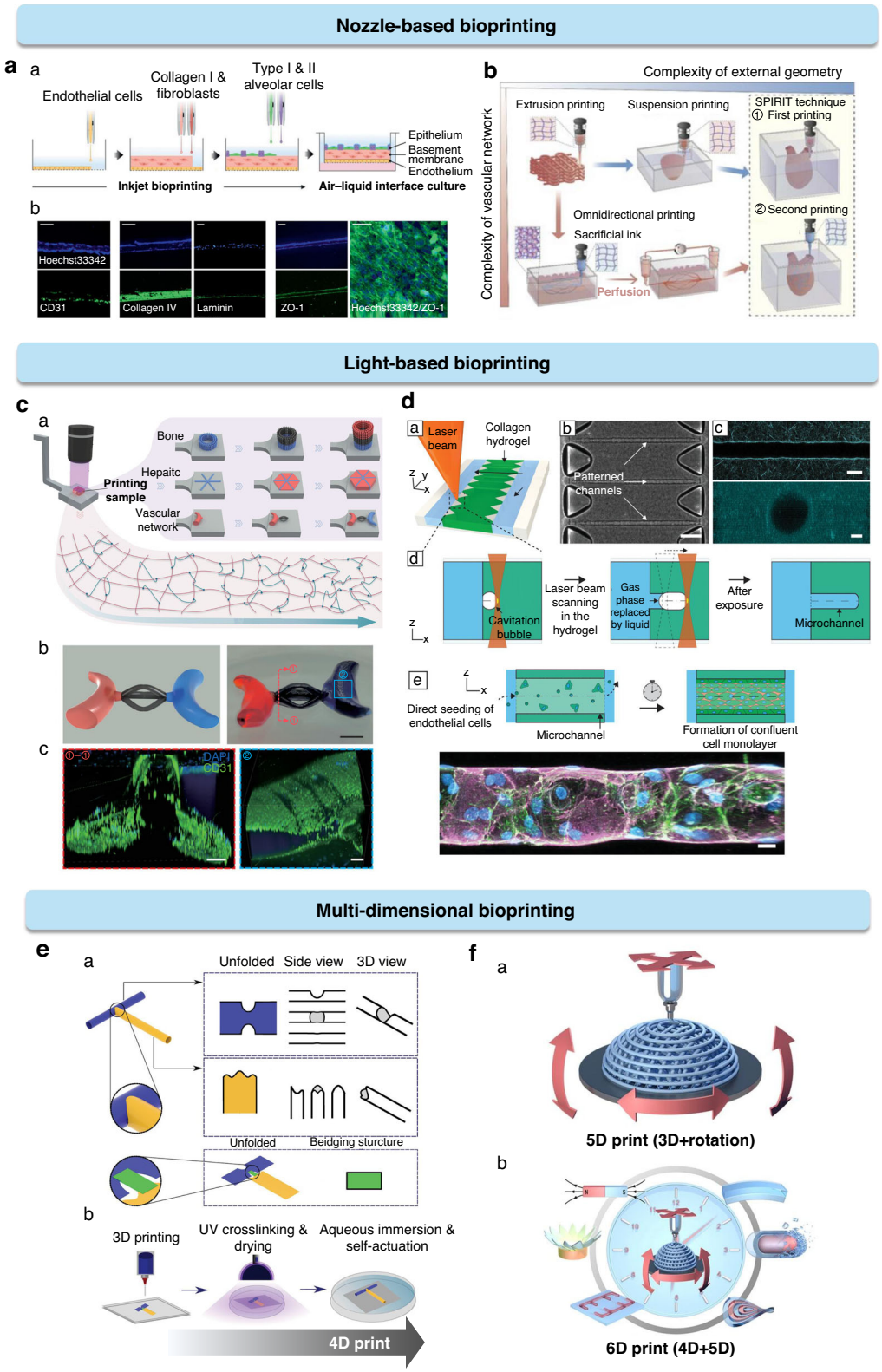

Fig. 4 (See legend on next page.)

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Fig. 4 Direct 3D bioprinting strategies for in vitro vascular microsystems. a Droplet-based bioprinting method for fabricating the alveolar barrier model. a Schematic of manufacturing process. b Immunofluorescence images of nuclei staining reagent Hoechst33342 (blue), the endothelial marker CD31 (green), the basement membrane makers collagen IV and laminin (green), and the tight junction marker ZO-1 (green). Scale bars: 20 μm[62]. Copyright 2021, Wiley-VCH. **b** SPIRIT strategy is developed by combining two embedded 3D printing methods. Two printing steps to engineering vascularized tissues and organs with complicated external geometries[69]. Copyright 2019, Wiley-VCH. **c** a Process illustration for multi-material DLP 3D bioprinting of heterogeneous structures and mechanical characteristics. b A STL file-based schematic design of a 3D vascular network model and the optical image of an actual printed sample of a multi-material 3D hydrogel vascular network. Scale bar: 2 mm. c Confocal fluorescence micrographs of ECs stained with CD31 and DAPI. Scale bar: 500 μm[73]. Copyright 2024, Wiley. **d** Direct laser pattering method for vascularized tissue models. a Schematic of in situ patterning of hydrogel by femtosecond laser exposure. b Brightfield image of patterned channels. Scale bar: 200 μm. c Confocal images of a patterned channel. Scale bars: 20 μm and 5 μm. d Diagram of the dynamics during laser-induced cavitation and after the collapse of the bubbles and matrix relaxation. e Conceptual drawing and confocal fluorescence image of direct seeding of ECs in the patterned channels. F-actin staining in green, platelet endothelial cell adhesion molecules-1 staining in magenta, and nuclei staining in blue, respectively. Scale bar: 20 μm[77]. Copyright 2022, Wiley-VCH. **e** Schematic representation of the fabrication of T-junctions: a the T-junction CAD design and b the 4D biofabrication process[81]. Copyright 2023, Wiley-VCH. **f** a, b The printing process for 5D and 6D printing[84]. Copyright 2024, Springer Nature

stress, which are common in nozzle-based printing methods[74]. This high-resolution and rapid printing capability is a significant breakthrough for in vitro vascular microsystems engineering, addressing the challenges related to the complexity and resolution of fabricated vascular networks.

Direct laser patterning Direct laser patterning varies from traditional layer-by-layer processing by facilitating in situ application through the interaction between focused lasers and materials. This interaction, which involves mechanisms such as melting, electronic excitation, and dissociation, allows for fine resolution and complex cellular distributions[75]. Hribar et al. reported an in situ 3D cell patterning technology in hydrogels. By utilizing gold nanorods embedded in a collagen hydrogel, which absorbed focused near-infrared femtosecond laser beams, the collagen locally denatures to form channels that allow cells to migrate, proliferate, and align themselves, ultimately forming a vascular-like channel. This technique showed high cell viability even at high writing speeds and low laser intensities, highlighting its potential for creating functional vascular microsystems[76]. On the other hand, Enrico et al. proposed a cavitation molding method that utilizes femtosecond laser irradiation to create microchannels and microcavities (diameters 20~60 μm). In this method, laser-induced cavitation gas bubbles rearrange collagen fibers to form microchannels, which are then seeded with endothelial cells to form vascularized tissue models (Fig. 4d)[77]. These breakthroughs in direct laser patterning address the need for fine control over the spatial organization of cells and the creation of highly detailed microstructures without compromising cell viability or material integrity[78,79].

Multi-dimensional bioprinting

The evolution of 3D bioprinting is currently advancing toward dynamic and functionally variable manufacturing, with the introduction of 4D, 5D, and even 6D bioprinting technologies. These advanced techniques extend 3D bioprinting by incorporating additional dimensions (time and information), enabling structures to adapt, respond, and change over time, and thus opening up new prospects for vascular microsystems engineering.

4D printing 4D printing expands the time dimension based on 3D bioprinting, enabling the manufactured components to change their structure over a period of time[80]. For example, Kitana et al. developed a method for creating self-actuated vascular bifurcations using 4D bioprinting technology. This method involved 3D-printed layers that changed shape upon immersion in water, transforming into a T-junction. The ability to manipulate the crosslinking time and other parameters allowed for the creation of vascular microsystems with dynamic features, such as varying diameters and self-actuation, which might have important implications for artificial blood vessels and vascular grafts (Fig. 4e)[81].

5D printing 5D bioprinting further extends the capabilities of 4D bioprinting by incorporating two additional axes of motion: the rotation of the print bed and movement along defined angles (Fig. 4f-a)[82]. The ability of 5D printing to produce curved, resilient surfaces and respond to external stimuli improves the strength and adaptability of vascular microsystems, making them ideal for studying vascularization in tissue engineering applications. It has the potential to create vascular microsystems that are both strong and flexible, capable of withstanding the mechanical forces present in vivo, such as shear stress and blood flow dynamics[83,84]. This makes 5D bioprinting particularly valuable for vascular disease modeling, drug screening, and the development of vascular grafts or prosthetics. Ruben Foresti et al. applied the 5D bioprinting method to create individual vascular models of patients. At 4D level, they designed bioinks and new composite materials to meet the specific functional

requirements. By tailoring the 4D model through the utilization of nanoparticles that engage directly with organ physiology, the 5D bioprinting model was obtained[85].

6D printing Although still in its conceptual stages, 6D bioprinting holds the potential to revolutionize multi-dimensional bioprinting by advancing beyond the capabilities of 4D and 5D printing. By integrating topological and textural modifications along with traditional motion axes, 6D bioprinting aims to further enhance the functionality of printed vascular microsystems (Fig. 4f-b)[83,84]. This additional level of complexity allows for the creation of vascular networks with exceptional precision, allowing them to dynamically respond to environmental changes, closely resembling the behavior of native tissues.

4D/5D/6D printing offer significant opportunities for constructing in vitro vascular microsystems: (1) Allowing for the creation of scaffolds that more closely mimic the intricate geometry of human blood vessels, providing new methods to construct complex biological structures; (2) The ability to print along additional axes (beyond the traditional X, Y, and Z) enhances the flexibility and accuracy of model construction; (3) Highly complex and curved structures with superior mechanical strength; (4) More material-efficient compared to 3D printing. However, several challenges need to be addressed for 4D/5D/6D printing to reach its full potential: (1) High cost for the additional axes and the specialized mechanical equipment; (2) Highly skilled expertise for the operation and maintain of these systems; (3) Software and hardware requirements for achieving the precision; (4) Difficulties in deformation control. Despite these challenges, the integration of time and information dimensions into bioprinting technologies holds unprecedented potential to revolutionize vascular microsystems engineering, thus paving the way for the development of more sophisticated, functional tissue and organ models for biomedical applications.

Supporting materials

In vitro vascular microsystems rely on supporting materials that serve as scaffolds, interacting directly with cells or biological tissues. These materials are designed to mimic vascular structures, creating a favorable microenvironment for vascular cell attachment and growth, closely resembling the physiological conditions in vivo. The design of supporting materials should balance mechanical strength, biocompatibility, and effective cell adhesion, while preserving cellular metabolic activity and ensuring that no undesirable adsorption interferes with the model's fidelity. Various biomaterials are employed in the design of vascular microsystems, with each material possessing unique properties that influence their application based on specific experimental goals (Table 1).

Biomaterials have promoted the development of in vitro vascular microsystems while making progress in a wide range of microfluidic fields. Key technical advancements in the development of supporting materials for vascular microsystems have focused on enhancing the ability to create flexible, biocompatible scaffolds that promote long-term cell viability and mimic in vivo tissue properties. For instance, surface modifications and novel polymer formulations have enabled improved cell culture environments, while the integration of different materials, such as elastomers with hydrogels, has been instrumental in addressing limitations like poor gas permeability and insufficient flexibility[86]. As in vitro vascular microsystems evolve, more emphasis is placed on selecting materials that not only support cell growth but also facilitate the simulation of complex physiological conditions, such as mechanical strain and gas exchange, which are critical for accurate vascular modeling[87].

In terms of the applications of in vitro vascular microsystems, the ideal supporting materials possess the following characteristics: (1) High biocompatibility-effectively promote cellular growth and proliferation; (2) Compatible mechanical properties-ensuring proper stress and strain responses; (3) Optical transparency-vascular cells can be readily observed; (4) Easy modification—more adaptable to cells; (5) Good gas permeability-the growth of vascular cells is dependent on gas exchange.

Glass

Historically, glass has been a primary material in microfluidic devices and remains widely used in early cell culture models[88]. Its optical transparency and biocompatibility allow for simple surface modifications to promote cell adhesion. However, the inherent limitations of glass, particularly its poor gas permeability and inflexibility, constrain its application in long-term culture and its ability to mimic the dynamic nature of vascular structures[89,90]. Due to the emergence of new materials, glass is no longer the sole primary material in cell culture platforms, and it is now commonly combined with other components to construct in vitro vascular microsystems, such as elastomers, thermoplastics, and hydrogels[91]. For example, hybrid microfluidic systems incorporating rigid glass with flexible materials are now used for vascular-liver co-culture models, facilitating the study of nutrient and drug metabolism (Fig. 5)[92]. Additionally, new fabrication methods, such as the integration of expandable glass with thermoplastic polymers, have streamlined the production of vascular microsystems, contributing to cost-effective, large-scale experimentation[93].

Table 1 Summary of supporting materials for in vitro vascular microsystems

Types	Materials	Advantages	Disadvantages	Fabrication methods	Refs.
	Glass	• Biocompatible • Optically transparency • Cells well adhere	• Poor gas permeability • High processing cost (Costly processing technology/expensive equipment)	• Laser ablation	92,93
Elastomers	PDMS	• Biocompatibility • Optically transparency • Flexibility • Ease of fabrication • Good permeability • Low cost • Chemical modification • Tunable mechanical properties	• Hydrophobicity • Nonspecific adsorption	• Templating molding • Micromilling • Soft lithography • Laser ablation	98,99
	FEPM	• Biocompatibility • Optically transparency • Low absorption • Flexibility • Chemical resistance	• Poor gas permeability • Expensive equipment		104
	PA	• Biocompatibility • Optically transparency • Flexibility • Low absorption • Tunable mechanical properties	• Incompatible with organic solvents • Complex surface treatment		106
Thermoplastic polymers	PMMA	• Optical transparency • Bioinertness • Low cost • Rigidity • Mass production	• Low permeability • Poor flexibility • Poor mechanical properties	• Micromilling • Laser ablation • Hot embossing • Injection molding	43,108
	PS	• Biocompatibility • Optical transparency • Low cost • Chemical inertness and stability • Rigidity • Mass production • Ease of modification	• Low permeability • Poor flexibility • Expensive equipment • Complicated process		116,117
	Thermoplastic elastomers	• Biocompatibility • Optical transparency • Tunable mechanical properties • Low absorption	• Poor resistance in specific solvents • Expensive equipment		122,123
Hydrogels	Natural hydrogels	• Naturally biocompatible and bioactive • Low cost • Ease of modification	• Easy degradation • Poor mechanical properties • Difficulty in tuning mechanical properties • Instability	• Templating molding • Laser ablation • 3D bioprinting	132–134,146,147,150,311–314

Table 1 continued

Types	Materials	Advantages	Disadvantages	Fabrication methods	Refs.
	Synthetic hydrogels	• Tunable mechanical properties • Ease of modification • Stability • Bioinertness	• Cytotoxicity • Relatively poor biocompatibility		
	Hybrid hydrogels	• Tissue-compatible mechanical properties • Biocompatibility • Tunable mechanical properties	• High processing cost • Complex experimental design		154–156

Elastomers

Elastomers typically consist of flexible polymer chains that are chemically bonded or held together by intermolecular forces. It has drawn considerable attention in the construction of in vitro vascular microsystem development due to their adaptability under mechanical strain and their ability to restore their original form after deformation[94]. Key technical breakthroughs in elastomer-based materials have enabled the development of more flexible and durable materials for vascular tissue engineering.

PDMS

PDMS is an extensively used elastomer material in organ-on-chip and microfluidic devices, serving as an essential foundation for in vitro models. Firstly, its plasticity and elasticity make it an ideal material for soft lithography. Early developments in soft lithography enabled the precise creation of microchannels for vascular cell culture, significantly advancing the ability to replicate blood vessel structures[95]. With its exceptional elasticity, PDMS flexible membranes are also widely used to simulate biomechanical strain in the blood vessel[96]. Secondly, due to its superior oxygen permeability (~2000–4000 $\mu m^2 s^{-1}$ [97]) and biocompatibility properties, it is highly suitable for long-term cell cultivation in closed chambers or channels. Thirdly, PDMS shows excellent optical transparency and can transmit ultraviolet and visible light, thus it is suitable for imaging. These inherent characteristics render it highly advantageous for applications in manufacturing, cell culture, and in vitro real-time analysis. For example, the development of PDMS-based models for vascular injury and bleeding studies demonstrated by Sakurai et al. has provided valuable insights into hemostatic mechanisms under controlled conditions[98]. Mao et al. developed a human-on-leaf-chip system with self-assembled 3D vasculature in a PDMS device, where VECs formed vascular networks under perfusion, mimicking the complex architecture of blood vessels[99].

However, PDMS has hydrophobicity, which poses a problem as many drugs of small hydrophobic molecules may combine with or be isolated within PDMS[100]. This characteristic can be addressed through chemical grafting and coating with hydrophilic materials, such as polyethylene glycol (PEG) or collagen. These advancements have enabled the production of more robust in vitro models, enhancing their application in drug screening, disease modeling, and vascular biology research[101]. Since PDMS is higher-cost than commonly used cost-effective polymers in the industry, the lack of efficient manufacturing and shaping methods impedes its large-scale production and commercialization[102].

Fluoropolymers

Fluoropolymers present a promising alternative to PDMS, owing to their C-F bonds that confer thermal stability and chemical inertness towards acids, bases, oxidizing and reducing agents, as well as most solvents[103]. Sano et al. developed a microchannel device with two layers of tetrafluoroethylene propylene monomer (FEPM) to apply mechanical strain on cultured VECs, replicating the epithelial-endothelial interface. They investigated the potential use of FEPM as an alternative material to PDMS to overcome its absorption of small hydrophobic molecules[104].

Polyacrylate (PA)

Polyacrylate exhibits comparable optical transparency, biocompatibility, flexibility, and replicability for cell culture as PDMS. However, it surpasses PDMS in its resistance to the absorption of small hydrophobic molecules[105]. Technical advancements in PA-based systems, such as the development of bifunctional coatings to enhance endothelial cell adhesion, have significantly improved the functionality of these models. The use of

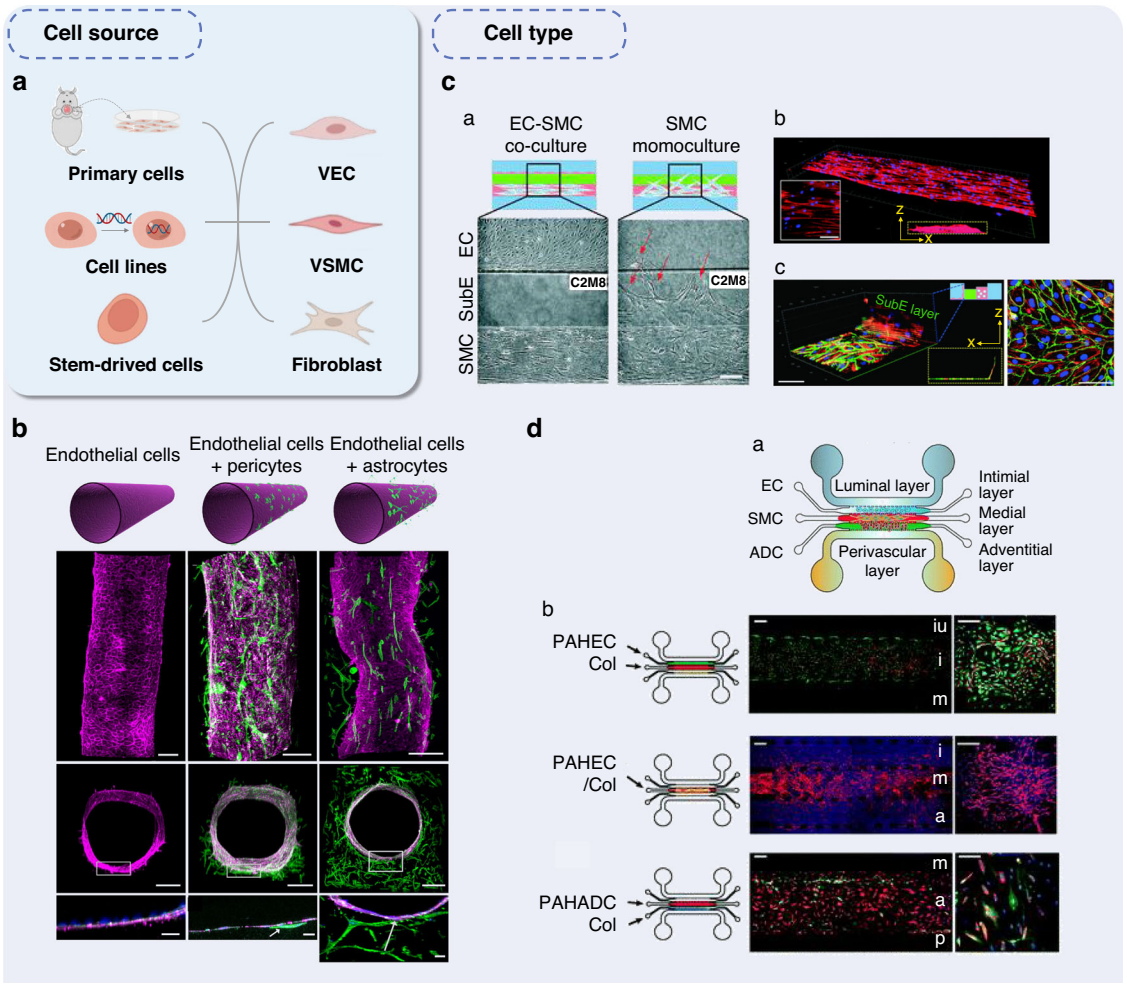

Fig. 5 Cells used in in vitro vascular microsystems. a Schematic of cell sources and cell types. **b** Schematic illustrations and immunofluorescence confocal images of VECs monoculture, VECs, and pericytes co-culture, as well as VECs and astrocytes co-culture. F-actin stained in green, the nucleus stained in blue, and VE-Cadherin stained in magenta. Scale bars: 200 μm and 30 μm[40]. Copyright 2016, Public Library Science. **c** Characterization of SMC phenotype under VEC and VSMC co-culture or VSMC monoculture. a Schematic and brightfield images of co-culture and monoculture. Scale bar: 200 μm. b 3D reconstructed fluorescence image of VSMCs co-cultured with VECs (F-actin stained in red, nucleus stained in blue). Scale bar: 100 μm. c 3D reconstructed fluorescence image and magnified image of VECs monolayer (VE-Cad stained in green, F-actin stained in red, nucleus stained in blue). Scale bar: 100 μm[174]. Copyright 2016, Royal Society of Chemistry. **d** A multichannel microfluidic device for co-culture of VECs, VSMCs, and adventitial cells to mimic the pathophysiology of PAH. a Schematic of the PAH-on-chip device. b Fluorescence micrographs of the growth of VECs, VSMCs, and ADCs in the device. Scale bars: 250 and 100 μm[187]. Copyright 2020, IEC & Royal Society of Chemistry & International Electrotechnical Commission

SLA to fabricate tubular structures with defined pores (~100 μm) has enabled the development of more sophisticated vascular models, which are capable of mimicking the architecture and dynamic behavior of blood vessels in response to flow conditions[106].

Thermoplastic polymers

Thermoplastic polymers are increasingly being used as supporting materials for in vitro cell culture models due to their low cost, high optical transparency, and rigid mechanical properties. These materials facilitate mass production through techniques such as hot embossing, injection molding, laser-induced cutting, and CNC milling, making them ideal for large-scale applications. Recent breakthroughs in the fabrication and modification of thermoplastics have particularly impacted vascular microsystem development, enabling more accurate modeling of vascular environments.

Polymethyl methacrylate (PMMA)

PMMA has found extensive applications in microfluidic devices, with several key advancements enhancing its

suitability for vascular modeling. While PMMA offers excellent optical transparency, bioinertness, and low cost, its application in cell culture requires additional surface modification for optimal cell adhesion and growth[107]. Surface treatments like UV treatment and poly(acrylic acid) (PAA) coating have been developed to improve PMMA's cell adhesion properties, addressing one of its main limitations[108]. Additionally, PMMA can be easily decomposed into methyl methacrylate at high temperatures, which is also known as "green microchips"[109]. These advances allow PMMA to be shaped via simple and cost-effective methods, such as hot embossing, injection molding, and micromilling, providing more accessible production techniques for vascular microsystems[110,111].

Polystyrene

As one of the most extensively utilized thermoplastics, PS possesses well-established applications in injection molding, which is also a standard material for cell culture consumable labware[112,113]. PS can be molded into microstructures with high replication fidelity, which is crucial for vascular modeling. PS has the characteristic of low absorbability toward small molecules, in comparison to PDMS, which has made it a preferred material for drug screening and toxicity studies. Moreover, its rigidity and optical clarity make it an excellent material for studying cell behavior, especially in controlled oxygen environments where its gas impermeability can be advantageous[114,115].

Recent innovations in PS microfluidics have demonstrated its potential for constructing in vitro 3D vascular networks. For example, Lim et al.[116] developed an injection-molded PS platform for studying hepatocellular carcinoma under hypoxic conditions, demonstrating the ability to use PS in modeling in vitro 3D tumor vasculature. Another significant contribution by Lee et al. was the use of PS for creating a 3D blood-brain barrier (BBB) model. This work illustrated the successful application of PS in addressing the limitations of PDMS-based microfluidic platforms, particularly in terms of manufacturing scalability and material compatibility[117]. While PS microfabrication remains challenging for low-volume academic applications due to its cost, the technology developments in PS processing have made it a viable alternative to PDMS for high-throughput and standardized vascular microsystems[118].

Thermoplastic elastomers

Thermoplastic elastomers (TPE) combine the beneficial properties of both thermoplastics and elastomers, making them highly suitable for the large-scale production of flexible vascular models. Their capacity to withstand dynamic mechanical strain while maintaining biocompatibility holds the potential to drive advancements in vascular tissue engineering. For example, simulating the vascular cyclic strain, aneurysm, and other dynamic studies can open up new opportunities in the study of vascular pathophysiology[101].

Styrene-ethylene-butylene-styrene (SEBS), a specific type of styrenic TPE, has shown exceptional promise for vascular models due to its combination of glassy thermoplastic characteristics and rubbery elastomeric properties[119]. The SEBS devices can obtain several appealing properties from the PS fraction, while the replication and fabrication processes can be simplified by the elastomeric properties[120]. It was demonstrated that vascular cells could grow on the SEBS substrates without surface treatments, and the rate of cell proliferation on these substrates was equal to that of commercial tissue culture. While subject to the condition of cyclic strain in blood vessel, SEBS's low elastic modulus (~1 MPa) and high stretchability (700–1000%) could be beneficial in the field of vascular tissue engineering[121].

Recent developments by Schneider et al. on hybrid microfluidic devices combining polycarbonate and TPE have further enhanced the optical properties and biocompatibility of these systems. This combination not only simplified fabrication but also ensured that microfluidic channels support cell growth without cytotoxicity, a critical breakthrough for organ-on-chip applications. These advances indicated that TPE can play a pivotal role in the next generation of vascular microsystems, particularly in drug testing and disease modeling[122].

Hydrogels

The above materials contributed outstandingly to constructing in vitro models and studying the physiology and pathology of blood vessels. However, their physical and chemical properties are far from natural ECM found in living organisms. Hydrogels have been extensively used in biomedicine for over a decade, providing promising applications for tissue engineering, drug delivery, and vascular modeling[123]. Hydrogels are described as two- or multi-component systems composed of polymer chains with 3D networks and water filling the gaps between macromolecules[124]. The interconnected pores in the hydrogels have high water retention, which is ideal for blood vessels as it allows for the efficient transportation of molecules from the blood to the tissues. In addition, hydrogels are available from a wide range of sources and are less expensive to produce[125].

While hydrogels have seen widespread use, their mechanical limitations and swelling properties pose challenges in the fabrication of precise vascular microsystems. The rheological properties and swelling behavior of hydrogels can distort microchannel structures, compromising model accuracy[126]. Additionally, hydrogels are sensitive to sterilization protocols, which may require

additional precautions during manufacturing[127]. Despite these limitations, hydrogels have proven invaluable, especially in simulating complex vascular microenvironments.

There are three main categories of hydrogels used in in vitro vascular microsystems: natural, synthetic, and hybrid hydrogels. The constituents of natural hydrogels containing native ECM proteins are similar to natural ECM, thus, exhibiting good chemical properties that are not inferior to native tissues. Synthetic hydrogels are bioinert and easily modified, and they present tunable mechanical properties. Hybrid hydrogels are proposed to merge the benefits of both natural and synthetic hydrogels and complement their shortcomings. However, the cellular microenvironments in living organisms are so complex that it is nearly impossible for any single hydrogel to replicate the functions of the natural ECM.

Natural hydrogels

Natural hydrogels, derived from ECM components such as collagen, fibrin, and hyaluronic acid (HA), are especially attractive for long-term culture of vascular cells due to their inherent biocompatibility and bioactivity. These hydrogels closely mimic the native tissue environment, promoting better cell adhesion, proliferation, and differentiation[128]. However, their use in long-term cell culture models can be challenging due to issues with adjusting their mechanical properties and degradation rates[129].

Collagen Collagen is widely used in vascular microsystems because of its ability to promote endothelial cell adhesion and support angiogenesis[130,131]. Recent researches, such as the use of rat tail collagen to model angiogenic sprouting in vitro, has led to more realistic vascular models[132,133]. Polacheck et al.'s work on perfused collagen-based microfluidic channels and the investigation of angiogenesis has significantly advanced the field of vascular microsystems, providing insights into vascular morphogenesis and cellular behavior under flow conditions. In this model, a profound study was conducted on the NOTCH1 signal pathway, which directly regulated the function of the blood vessel barrier and facilitated the assembly of adhesion connections[134].

Hyaluronic acid HA is a natural glycosaminoglycan as well as an essential component of the ECM. It has shown significant potential in promoting vascular cell proliferation and angiogenesis[135]. Its ability to be modified to enhance material properties makes it a valuable tool for tissue engineering[136]. Recent advancements have focused on the manipulation and modification of the properties to create hydrogels with enhanced mechanical strength, supporting more durable and long-term vascular models[137].

Gelatin Gelatin, derived from collagen, offers an excellent option for vascular modeling due to its biocompatibility and easy modification for biofabrication. Unmodified gelatin is unstable in physiological conditions and has poor mechanical properties. A stable photopolymeric hydrogel at 37 °C can be obtained by the chemical modification of the gelatin side groups. It can quickly form within seconds through UV-crosslinking, making it a popular material for biofabrication[138]. Gelatin with temperature-sensitive properties can also be utilized as a sacrificial material for creating hollow channels[139]. Gelatin methacryloyl (GelMA), as a derivative of gelatin, has been used in combination with 3D bioprinting techniques to fabricate vascularized tissue models with enhanced mechanical properties, making it a promising material for tissue engineering. Shao et al. utilized GelMA/gelatin as the bioinks to develop a new direct coaxial 3D bioprinting strategy to create a vascularized structure consisting of a core (gelatin)-sheaths. This structure can achieve self-supporting and meet the demand for mechanical properties in the 3D cell culture[140]. Besides, Tang et al. have developed an aqueous two-phase embedded bioprinting approach based on GelMA and poly(ethylene oxide) that can be utilized as the aqueous support bath to fabricate standalone cannular tissues with ultrasmall luminal diameters and ultrathin walls[141].

Synthetic hydrogels

Synthetic hydrogels, which are made from non-natural molecules, show improved consistency and tunability in terms of composition and material characteristics compared to natural hydrogels. While these hydrogels lack integrin and growth factor binding sites essential for cell adhesion and function, these can be incorporated through the addition of bioactive molecules such as proteins, enzymes, and growth factors, further improving cellular interactions[142]. Additionally, synthetic hydrogels can be easily modified to control porosity, mechanical strength, and biocompatibility, allowing for precise tailoring to specific vascular microsystem applications.

PEG-based hydrogels PEG-based hydrogels are the representative synthetic biomaterials in cell culture and tissue engineering because they are non-toxic and non-adhesive, and they can provide adequate hydration and nutrients[143]. Poly(ethylene glycol) diacrylate (PEGDA), a modified PEG, contains acrylate groups that enable rapid polymerization upon exposure to light[144]. This modification allows for precise control over the mechanical properties and crosslinking kinetics, facilitating the creation of stable 3D hydrogels[145]. Using PEGDA, Zhang et al. created perfusable vascular-like networks and

culture chambers by SLA. These networks, connected to an external pumping system, demonstrated the ability to sustain long-term cell cultures in a dynamic environment, mimicking the nutrient and oxygen transport found in natural blood vessels[146]. Furthermore, PEG-based hydrogels were used to fabricate bicuspid venous valves that exhibited fluid mixing and bicuspid valve functionality, showing the flexibility and adaptability of PEG hydrogels in functional tissue modeling. The straightforward axial channel surrounded by a helical channel structure demonstrated the feasibility of intervascular oxygen transport within 3D hydrogel networks[147].

Polyacrylamide Polyacrylamide (PA) hydrogels, synthesized by polymerizing acrylamide monomers, offer an advantage in mechanobiology by providing precise control over hydrogel stiffness, a crucial factor in modeling blood vessel rigidity[148]. The unique characteristics of PA-gel make it a commonly utilized material in the construction of in vitro vascular microsystems, particularly for studying the effects of the change in the blood vessel rigidity on the various cellular functions within the vasculature[149]. Shin et al.[150] used PA-gel to mimic endothelial dysfunction. By adjusting the stiffness of the PA-gel by modifying the ratio of acrylamide solutions and cross-linker, they were able to replicate specific factors found in early atherosclerotic plaque lesions. This customization of stiffness allows PA hydrogels to serve as an effective tool for studying the mechanical influences on vascular cells and their roles in various diseases.

Hybrid hydrogels

With the development of manufacturing techniques for in vitro models, the limitations of single hydrogels are progressively revealed and may not adequately fulfill the requirements for device manufacturing and functionality. Combining multiple natural hydrogels to form hybrid hydrogels can enhance their advantages and mitigate their respective disadvantages. The hybridization of hydrogels can occur between natural-natural, synthetic-synthetic, and natural-synthetic hydrogels.

Natural-natural hybrid hydrogels By mixing two or more natural polymers, these hydrogels aim to mimic the composition of native ECM more accurately. These hydrogels can replicate the cellular affinity and biocompatibility of natural ECM while maintaining better structural integrity compared to single natural polymers[151].

Synthetic-synthetic hybrid hydrogels They are produced by copolymerizing two or more synthetic polymers. These combinations result in improved mechanical properties and compatibility, which are essential for producing stable, functional vascular models[152].

Natural-synthetic hybrids Natural-synthetic hybrids have become particularly valuable in in vitro vascular microsystems. While natural hydrogels provide excellent cell affinity, synthetic hydrogels allow for better control over degradation rates, mechanical strength, and water content. The combination of these two types of hydrogels has significantly improved the reliability and functionality of vascular models used in cell culture[153].

A noteworthy example is the hybrid microfluidic platform developed by Paek et al.[154], which incorporated fibrin and type I collagen hydrogels to support the formation and anastomosis of 3D vascular networks. This system enabled the integration of multiple cell types and replicated the cellular diversity and structural arrangement of vascularized human tissues found in organs. A perfusable, endothelialized vascular chip was designed featuring an agarose-gelatin interpenetrating-polymer-network hydrogel that can tune the stiffness to approximate that of the blood vessel intima[155]. This adjustment enhanced the realism of the engineered microvasculature and promoted the endothelial-barrier function to mimic physiological conditions. Furthermore, Gold et al. demonstrated the creation of 3D-printed vascular structures using a GelMA, PEGDA, and nanosilicate composite hydrogel, which maintained tunable mechanical properties and supported the co-culture of VECs and VSMCs. This advancement facilitated the prediction of human thrombo-inflammation, showcasing the capacity of hybrid hydrogels to model complex vascular responses to external stimuli[156].

Cell type

Physiological blood vessels rely on the coordination and functioning of multiple types of cells and ECM. Biomaterials can mimic the ECM microenvironment, offering higher potential for cellular and tissue growth and development. Cells can be considered the “soul” of in vitro biological models, responsible for executing essential biological functions. Therefore, the incorporation of cells is crucial in creating in vitro vascular microsystems with elemental biological functions. The selection of cells primarily involves considering both their sources and types (Fig. 5a).

Cell sources

There are three main sources of cells utilized in in vitro vascular microsystems: primary cells, cell lines, and stem cell-derived cells. The majority of these models heavily rely on the primary cells and cell lines.

Primary cells

Primary cells are specialized cells that can be differentiated from a specific tissue or organ, maintaining their original genome and closely resembling the characteristics and functions of the native tissue[157]. These cells, such as VECs isolated from the human umbilical vein, VSMCs isolated from the aorta, and fibroblasts isolated from the dermis, are the most suitable cells to mimic the in vivo state and physiology in the in vitro models. However, the restricted availability of human primary cells and the difficulties associated with their cultivation present significant challenges, such as specialized growth conditions and batch-to-batch variability. Despite these limitations, primary cells remain crucial for accurately modeling vascular systems in vitro. The innovations in cell sourcing and expansion techniques, such as improved cell culture methods and the use of microfluidic platforms, have increased the availability and reproducibility of primary cell-based models. These advancements have facilitated a more consistent and long-term use of primary cells in vascular modeling[158].

Immortalized cell lines

Immortalized cell lines refer to cancerous cells that undergo continuous division or cells that have been genetically modified to proliferate indefinitely. These cells can be cultured for multiple generations, providing advantages in terms of cost, manipulation, and expansion[159]. Additionally, the use of immortalized cell lines avoids ethical issues related to the use of animal and human tissue. Immortalized cell lines derived from different tissues exhibit the distinct features of tissue functions and provide a homogeneous population, which is useful for providing a consistent sample and reproducible results. Furthermore, they serve as valuable tools for constructing in vitro models of diverse tissues for physiological and pharmacological investigations[160,161]. VECs, VSMCs, and fibroblasts from various anatomical regions can be immortalized. However, during the process of infinite proliferation, these cells may undergo a loss of tissue-specific functions and physiological properties, ultimately leading to they may not fully recapitulating the complex behaviors of native vascular tissues[162].

Stem cell-derived cells

When primary cells or cell lines fail to achieve the desired effects, stem cell-derived cells, including embryonic stem cells (ESC), induced pluripotent stem cells (iPSCs), and mesenchymal stem cells, *etc*, can be promising alternatives[163]. iPSCs are reprogrammed from somatic cells to ESC-like pluripotent states, enabling the generation of large quantities of individual-specific cells[164]. iPSCs derived from both healthy individuals and patients can yield various types of vascular cells, allowing for the replication of individual variations. This has significant applications in in vitro vascular microsystems, as well as in mechanism research, drug screening, and personalized medicine development[165]. iPSCs can be considered as the optimal cellular source for in vitro modeling in the future[166]. Despite the apparent advantages of conducting biological studies in vitro, there are still limitations associated with these cells, such as low reprogramming efficiency, genetic instability, and a lack of universally acceptable assessment criteria[167].

Cell types

In addition to selecting the appropriate cell sources, cell types cultured in the model should also be considered. It is worth noting that while blood vessels in vivo share common characteristics, such as cell types and subtypes, there can be significant variations in vascular structure and functions among different tissues. For instance, studies focusing on arteries should select arterial endothelial cells, whereas studies of veins require venous endothelial cells. When constructing cerebral vascular tissues, it is crucial to consider the induction effect of pericytes and astrocytes on endothelial cells[168]. Similarly, the blood vessels in the pulmonary cannot neglect the crosstalk between epithelial-endothelial-fibroblasts[169]. According to the research purposes, monoculture can be selected to investigate a specific cell type and simplify the model, or multiple cell co-culture to construct more complex tissues and organs, thereby improving the reliability and relevance of the model[170]. Blood vessels primarily consist of VECs along with supporting cells, including VSMCs, pericytes, and fibroblasts[171].

VECs

VECs are the predominant vascular cells, lining the interior surface of blood vessels in a single-layered and squamous form. Extensive research has focused on VECs in studying vascular physiological systems, considering their variations across different developmental stages, as well as different tissues and organs. For instance, VECs at the embryonic stage show a non-specialized endothelial phenotype. Moreover, the functions of VECs in cerebrovascular and cardiovascular systems also have dissimilarity, due to specific physiological environment conditions and epigenetic modifications[172]. VECs generally have special organelles (Weibel-Palade body), and express specific vascular endothelial markers including platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31), intracellular adhesion molecule-1, vascular cell adhesion molecule-1, and selectins, etc[173]. VECs are commonly used in in vitro vascular microsystems, including human umbilical vein endothelial cells (HUVECs), human microvascular endothelial cells (HMVECs), and human aortic endothelial cells

(HAECs)[174]. In addition, non-human cells such as bovine aortic endothelial cells) are also employed[150]. HUVECs are derived from umbilical cord tissues that are easy to isolate and culture in vitro, and can generate stable vascular channels or networks. As the most extensively used VECs, HUVECs show general and representative characteristics with essential endothelial markers[175]. However, it is worth noting that HUVECs represent only certain stages of human life and serve as a general model for VECs both in normal and diseased conditions. However, they may not express certain tissue markers[176]. On the other hand, HMVECs are originally obtained from microvessels, so they have great potential to form microvascular networks. Using a 3D microfluidic platform, Seo et al. compared the angiogenic capabilities of HAECs and HUVECs, revealing distinct expression patterns of certain angiogenesis-related factors between these two cell types[177].

HMVECs isolated from capillaries of different tissues can express specific endothelial properties and reproduce tissue-specific vascular systems, such as human brain microvascular endothelial cells (hBMECs)[178], human pulmonary microvascular endothelial cells[179], and human dermal microvascular endothelial cells[180]. These cell types demonstrate excellent proliferative capacity in vitro. Recent developments in microfluidic platforms have enabled more sophisticated models of endothelial function. For example, 3D models using hBMECs have been successfully utilized to replicate the BBB, offering new insights into neurovascular inflammation and drug delivery (Fig. 5b)[40]. Furthermore, advances in co-culture techniques have allowed for the study of endothelial cell behavior in the context of other cell types, such as pericytes, which play a critical role in vascular integrity and angiogenesis[30,181].

VSMCs

Monolayer endothelialization alone may not suffice for in vitro vascular modeling, as the crosstalk between VECs and mural cells (VSMCs and pericytes) is crucial for the optimal functionality of most blood vessels. VSMCs, which constitute the primary cell types of the tunica media, play a vital role in maintaining vascular wall integrity and regulating blood pressure. They interact with VECs to control the development and remodeling of vessel wall structures necessary for maintaining mechanical homeostasis.

VSMCs differ from other muscle cells in that they have a contractile phenotype or a synthetic phenotype. In healthy arteries, contractile proteins are highly expressed in VSMCs, including α-smooth muscle actin (αSMA/ACTA2), smooth muscle cell myosin heavy chain (SMMHC/MYH11), and Transgelin (SM22α/TAGLN)[182]. VSMCs play a critical role in developing and progressing many vascular diseases, such as AS, Inflammation, and aortic aneurysm[183]. For example, Su et al.'s microfluidic EC-SMC platform successfully replicated early AS and identified key ECM compositions critical for maintaining VSMC behavior (Fig. 5c)[174]. This platform held significant promise for drug screening and disease modeling.

Fibroblasts

Fibroblasts are indispensable for synthesizing and maintaining the ECM and are implicated in crucial processes like angiogenesis and fibrosis. These cells are particularly important in vascular microsystems, as they interact with VECs and contribute to vessel stability and remodeling[184]. Fibroblasts seeded in in vitro vascular microsystems can be characterized with common markers, including fibroblast-specific protein-1, collagen type I, as well as the cell surface receptors CD34 and platelet-derived growth factor receptor, etc.

In addition, fibroblasts are necessary for the tumor microenvironment[185]. For instance, Lai Benjamin et al. built a 3D vascularized model of the pancreatic tumor using a tri-culture system consisting of patient-derived pancreatic organoids, human fibroblasts, and VECs. The investigation of the tumor microenvironment revealed that the collaborative interaction between fibroblasts and patient organoids resulted in a remarkable augmentation of collagen deposition and tissue stiffness[186]. Moreover, Al-Hilal et al. cultured three different types of cells found in the pulmonary arteries—VECs, VSMCs, and adventitial cells (primarily fibroblasts), in order to fabricate a device emulating the pathophysiology of pulmonary arterial hypertension (PAH). The objective of this device was to replicate the main pathological features of PAH in vivo (Fig. 5d)[187].

Geometry of in vitro vascular microsystems

Cells within 3D microenvironments are organized based on vessel geometry, which significantly influences cell functions. Drawing the support from current advanced fabrication technologies, it is now feasible to easily create regular geometries (e.g., stenotic, bulging, tortuous, spiral, and bifurcating), as well as irregular geometries (e.g., networks)[68]. The pathogenesis of diverse blood and vascular disorders entails aberrant interactions among different types of blood cells and vascular cells, which are significantly influenced by the hemodynamic forces exerted by the local morphology and architecture of blood vessels. Therefore, it is necessary to investigate the physiological and pathological reactions of blood vessels in specific regions within the context of vascular geometries. In the vasculature-rich tissues and organs, reduplicating vessel geometries are closer to a fully reconstructed cell environment in vitro.

Stenosis

Vascular stenosis is a pathological hallmark of AS, leading to severe cardiovascular disorders like myocardial infarction and stroke. The channel with varying degrees of stenosis can also be utilized to evaluate how wall shear stress conditions affect cells based on the specific geometry of the narrowing[188]. For example, Chen et al. constructed a round deformable soft microfluidic channel to recapitulate AS with 3D stenosis. The model has variable radii with circular cross-sections that can reproduce physiological-mechanical stimulations, such as pulsatile pressure, fluid shear force, and cyclic stretching (Fig. 6a)[189]. Deng et al. conducted a study in which they integrated an optical time-stretch imaging system into a microfluidic 3D stenosis model. enabling the observation of platelet aggregation caused by stenosis under different spatial and temporal conditions. The study ultimately revealed the combined impact of atherogenic blood flow disturbance and the agonist on platelet activation[190]. Further, they evaluated the efficacy of antiplatelet drugs in the stenosis vascular model, which contributed to the personalized medical treatment strategies for atherosclerotic patients (Fig. 6b)[191].

Bulging

Bulging models are commonly employed in vascular modeling to simulate diseased blood vessels and examine their flow dynamics. Aneurysm, characterized by balloon-like bulge on the wall of the artery, presents a more intricate physicochemical microenvironment. Given the high cost and technical challenges associated with animal studies, in vitro models that mimic aneurysm geometry offer a valuable means to generate flow patterns more akin to those occurring in vivo, facilitating the study of cell phenotypes and inflammatory mediators[192].

More recently, Yong et al. developed a 3D intracranial aneurysm model based on patient-specific vascular dimensions to simulate fluid flow and predict aneurysm behavior under various conditions, providing a platform for studying aneurysm pathophysiology and testing treatment strategies (Fig. 6c)[193]. In another study, Xie et al. created a 3D glomerular filtration barrier model, simulating kidney microvascular environments, and demonstrating the influence of blood vessel geometry on podocyte function and barrier integrity (Fig. 6d)[194].

Tortuous and spiral

Tortuous or spiral blood vessels, which are common in specific areas, experience changes in mechanical forces due to their unique geometry. Unlike straight vessels, tortuosity alters shear stress, pressure distribution, and endothelial behavior, thereby necessitating the development of in vitro models that capture these biomechanical nuances[195]. Recent progress in bioprinting techniques has enabled the creation of tortuous vessel models that replicate these geometric changes. For example, in a 3D vascularized proximal tubule (PT) model of adjacent conduits that were lined with confluent epithelium and endothelium, lumen geometry and fluidic shear stress significantly enhanced epithelial cells maturity over time in vitro[196]. Park et al. used 3D triaxial bioprinting with a brain-specific hydrogel bioink to create cerebrovascular conduits with varying curvatures. These models showed that higher vascular curvature regions experience reduced shear stress and velocity, influencing the molecular signatures of metastatic potential. This study demonstrates the significant role of geometry in regulating vascular responses to fluid dynamics and tumor cell interactions (Fig. 6e)[197].

Spiral microvessels are integral to multiple organ systems, including endometrial spiral arterioles, intestinal villi spiral arterioles, and cochlear spiral modiolar arteries. In vitro models of spiral vascular offered a novel research platform for investigating the reactions of helical blood vessels to structural and environmental changes[198]. Mandrycky et al. developed a spiral microvessel system with precisely controlled curvature and torsion, demonstrating that small, twisted vessels exhibited distinct endothelial phenotypes and transcriptional profiles compared to straight vessels. This finding provided valuable insights into the influence of vascular geometry on the heterogeneous vascular response to flow (Fig. 6f)[199]. Another typical study came from Grigoryan et al., their serpentine-helix model demonstrated the feasibility of intervascular oxygen transportation within 3D entangled networks[147].

Bifurcating networks

The bifurcating vascular networks are typically present in physiology with specific hemodynamic characteristics. Branching structures constructed in vitro have been used to evaluate the effect of bifurcated fluid flow and laminar shear stress on vessel permeability[200]. Integrating direct current electric fields, researchers yielded valuable findings concerning the role of the endothelium as an electromechanical interface for the regulation of vessel permeability, which is significant for tissue regeneration and wound healing[201]. Vascular systems in the body mostly exist in multi-branched network structures. Engineering of vasculature network models with branches at the vasculature size scale is more appropriate for disease modeling in a specific location, such as coronary artery bifurcation disease (Fig. 6g)[202].

Advanced techniques like embedded 3D printing are instrumental in achieving geometrically bifurcating and intricate structures. The vascular network-inspired fluidic system (VasFluidic) developed by Yu et al.[203] replicated

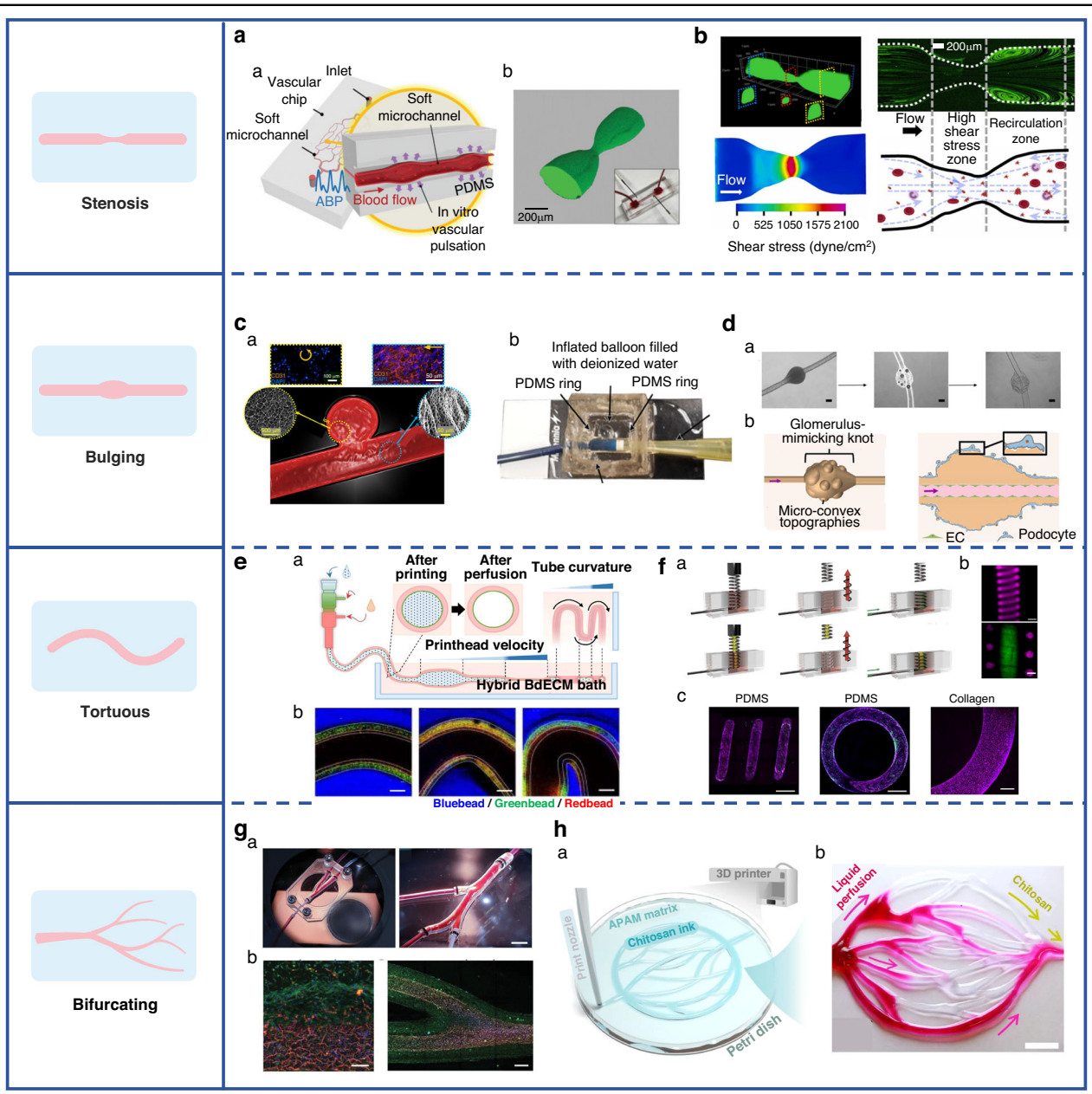

Fig. 6 Various geometries of in vitro vascular microsystems. a a Schematic of the vascular chip with soft microchannels. b Fluorescence images and photographs of chip with stenosis channel[189]. Copyright 2024, Wiley. **b** (Top left) Fluorescence image of the stenosis model filled with fluorescence solution. (Bottom left) Shear stress simulation at an inlet wall shear stress (WSS) of ~20 dyne/cm^2. (Top right) Images of perfusing of FITC-conjugated 2 μm beads in whole blood at an inlet WSS of ~20 dyne/cm^2. (Bottom right) The atherogenic flow profile and the process of platelet aggregation within the microchannel[191]. Copyright 2023, Royal Society of Chemistry. **c** A 3D IA model with bulging structure. a VECs marker staining in red and nucleus staining in blue in the vessel and bulge regions after perfusing. Scanning electron microscopy (SEM) images of the hollow GelMA hydrogel IA model. b Image of the structure of IA model[193]. Copyright 2021, Elsevier. **d** a The formation of the microconvex topography on the hydrogel knot surface. b Design of the structure of the alginate fiber with a glomerulus mimicking knot; VECs and podocytes seeding on the channel and knot[194]. Copyright 2020, American Chemical Society. **e** The fabrication of geometrically controllable vascular conduits. a Using a triaxial nozzle, hybrid brain-derived extracellular matrix (BdECM) bioink, and calcium-added Pluronic F-127 (CPF-127) and controlling diameter and curvature of the conduits by managing pneumatic pressure, printhead velocity, and programming commands. b Tubes with different angles. Scale bars: 500 μm[197]. Copyright 2023, Springer Nature. **f** In vitro vascular model with spiral structure. a Diagram illustrating the fabrication strategy for the spiral vessel. b Fluorescent images of a spiral vessel in collagen perfused with fluorescent beads. Scale bar: 500 μm. c Fluorescent images of side and top views of an endothelialized spiral vessel in PDMS and top view of endothelialized collagen vessel. Scale bars: 750, 600, and 150 μm)[199]. Copyright 2020, American Association for the Advancement of Science. **g** a Photographs of vascular constructs with branches. b Confocal image of bifurcating vascular construct, α-SMA staining with green and CD31 staining with red. Scale bar: 100 μm and 500 μm[202]. Copyright 2022, Wiley. **h** a The printing process of vascular channels. b Infusion of liquids into a vascular network-shaped channel after removing matrix. Scale bars: 1 cm[203]. Copyright 2024, Springer Nature

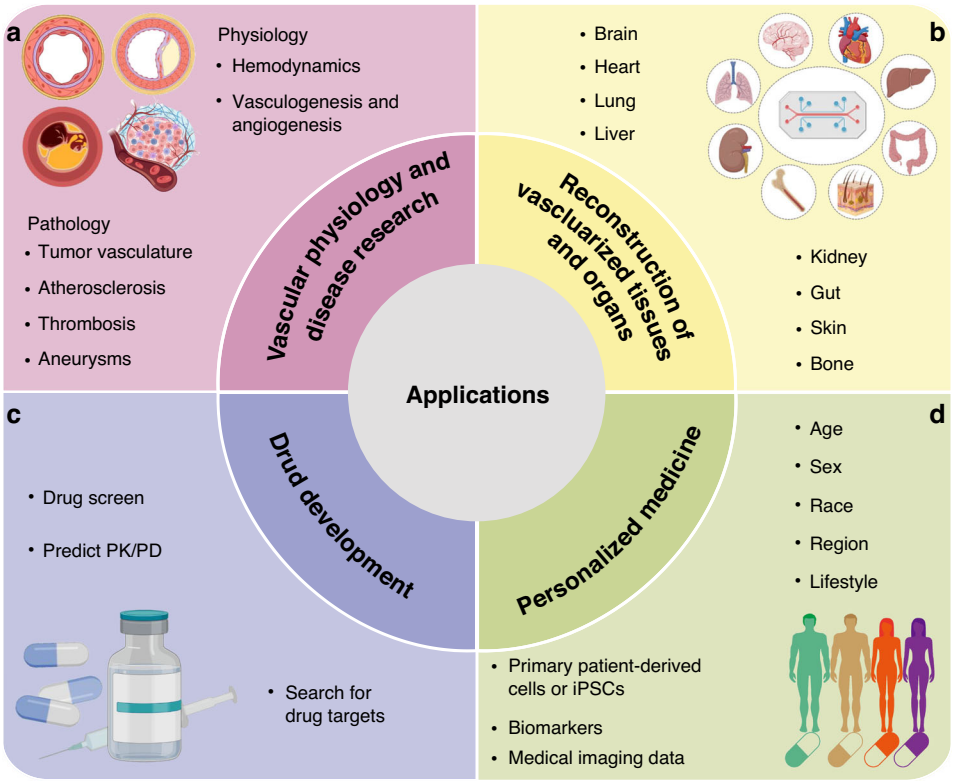

Fig. 7 Applications of in vitro vascular microsystems in biomedical research. a Investigation of vascular physiology and pathological mechanisms in diseases. **b** Reconstruction of vascularized tissues and organs for regenerative medicine. **c** Applications in drug screening and development pipelines. **d** Advancements in personalized medicine through patient-specific modeling. All schematic illustrations were created with BioRender.com

the flow in blood capillaries with a diameter of 7–9 μm, which was crucial for modeling the complex geometry of natural vascular systems (Fig. 6h). The engineered vasculature-rich tissues and organs are also conditional on generating 3D vascular networks that sustain the viability of parenchyma cells. Rayner et al. described an in vitro reconstruction of the renal vascular-tubular exchange unit, wherein a device was developed to integrate renal vascular channels with grid geometry and tubular channels with parallel geometry. This model facilitated investigations into renal microcirculation and endothelial-epithelial interactions[204]. Moreover, gridded and multi-branch vascular networks are also commonly used to fabricate pre-patterned, perfusable implants to establish rapid blood perfusion and efficient anastomosis in vitro[205,206].

Applications of in vitro vascular microsystems

As materials and technology develop, in vitro vascular microsystems have been applied to various fields, showing great potential for a broad range of vascular biological and medical studies. Here we elucidate the application of in vitro vascular microsystems in four key areas: vascular physiology and pathology research, reconstruction of vascularized tissues and organs, drug development, and personalized medicine (Fig. 7). The applications of engineered in vitro vascular microsystems described here, engineering approaches, and features are summarized in Table 2.

Vascular physiology and pathology research

Significant breakthroughs have been achieved in the development, pathogenesis, and drug therapy of vascular diseases, depending on animal models and 2D cell culture. However, with the long-standing limitations of traditional research systems and advances in tissue engineering and model fabrication technologies, in vitro models have emerged as indispensable tools for investigating vascular physiology and pathology.

Physiology

Engineering hemodynamic microenvironments A complex vascular mechanical microenvironment exposes VECs to a series of hemodynamic forces, such as fluid shear stress, cyclic stretch, and lateral pressure. A central challenge in vascular modeling has been replicating the

Table 2 Applications of engineered in vitro vascular microsystems

Applications		Structure and design	Refs.
Vascular physiology	Hemodynamic microenvironments	• 3D microfluidic model with physiological flow/strain for VEC-VSMC co-culture.	208
		• Microfluidic vascular model with stretchable electrochemical sensors.	209
	Vasculogenesis/angiogenesis	• Collagen gel-embedded microvessel with angiogenic factor gradients.	212
		• Endothelium-like structure with proangiogenic agents.	213
Vascular pathology	Tumor vasculature	• 3D bioprinted vascularized tumor microenvironment with breast cancer cells.	217
		• 3D bioprinted artificial tumor with autonomous endothelial/stromal cell organization into perfusable vasculature.	218
	Atherosclerosis (AS)	• Microfluidic model with cyclic stretch and fluid shear stress on VECs.	222
		• Coronary artery model with low/oscillatory shear stress on VECs.	223
	Thrombosis	• 3D-printed intracranial aneurysm model with live endothelial cells.	227
		• PDMS chip with dual channels (vascular and anticoagulant port).	229
	Aneurysms	• CFD-informed microfluidic channels with site-specific flow dynamics.	233
		• 3D-printed patient-specific aneurysm model with live endothelial cells.	235
		• MRI-compatible transparent vessel-on-a-chip with dynamic flow monitoring.	236
Vascularized tissues/ organs	Brain	• Neurovascular-unit-on-a-chip (neural stem cells, endothelial cells, pericytes).	241
	Heart	• Heart-on-chip with cyclic mechanical strain.	246
	Lung	• Sandwich structure with alveolar (air) and vascular (liquid) channels and a flexible membrane. Multifunctional microdevice with epithelial-VEC tissue interface, ECM matrix, and mechanical stretching capability.	248
		• Flexible PDMS device with cyclic suction chambers and engineered alveolar-capillary interfae.	250
	Liver	• Microfluidic liver sinusoid model with separate chambers for hepatocytes (collagen-embedded) and endothelial cells.	255
		• Species-specific liver on chip with ECM-rich upper parenchymal channel (hepatic cells) and lower vascular channel.	256
	Kidney	• Microfluidic renal PT model with epithelial-VEC co-culture and peritubular capillary simulation	259
		• Glomerulus-on-chip with iPSC-derived podocytes, glomerular endothelial cells on PDMS membrane, and side chambers for pulsatile mechanical strain	260
	Gut	• Dual-channel design (intestinal and vascular lumen) with porous membrane.	264
	Skin	• Endothelial cell seeding and perfusion.	268
		• 3D bioprinted skin with perfusable vascular channels in dermal/hypodermal layers.	269
	Bone	• Bone marrow-on-a-chip (fibrin gel and vascular channel).	272
Drug development		• Engineered tumor vascular network with perfusion.	276
		• Interconnected organ chips (gut-liver-kidney) via vascular channels.	277
Personalized medicine		• Glomerular filtration barrier model with patient-derived cells.	282
		• iPSC-derived BBB organ-on-chip.	284

intricate hemodynamic forces that regulate cell behavior[207]. Recent engineering advancements have enabled the development of microfluidic devices capable of applying precisely controlled mechanical forces with high accuracy and reproducibility. For example, van Engeland et al. constructed a 3D microfluidic model to replicate VEC-VSMC niche, incorporating physiological flow and strain conditions. This model successfully simulated the

cellular arrangement and composition, demonstrating its applicability for investigating the interaction between VECs and VSMCs in physiological and pathophysiological hemodynamic loading conditions[208]. To monitor the mechanical force-triggered signals in situ and in real-time during vascular mechanotransduction, Jin et al. developed a microfluidic vascular model that incorporated a flexible and stretchable electrochemical sensor. This innovative model not only replicates physiological characteristics of blood vessels in vivo, but also provides real-time monitoring of mechanically induced biochemical signals[209].

Modeling vasculogenesis and angiogenesis Reproducing the formation of new blood vessels from endothelial precursors (vasculogenesis) and the sprouting of vessels from preexisting ones (angiogenesis) has been another major challenge. In vitro vascular microsystems serve as powerful tools for observing and manipulating the progression of vasculogenesis and angiogenesis, enabling researchers to examine the effects of various extracellular regulatory factors, including growth factors, cytokines, and adhesion molecules. By generating stable gradients of angiogenic factors and incorporating co-culture systems with supporting cells, engineered microsystems now closely replicate the dynamic vascular self-assembly seen in vivo[210,211]. For example, Pauty et al. developed an in vitro model that embedded an initial microvessel within a collagen gel. They have not only demonstrated the critical role of the Notch signaling pathway in sprouting angiogenesis but also provided reproducible platforms for drug testing and mechanistic studies[212]. Similarly, Dikici et al. fabricated an endothelium-like structure to investigate the migratory response and tube-forming ability of VECs, in the presence of proangiogenic agents[213]. This study aimed to establish a reliable in vitro model for studying tissue-level vascularization. These technical developments have shifted vascular research from static observations to dynamic, controllable experiments that yield deeper insights into vascular development and remodeling.

Pathology

Damage and disease of the blood vessels can give rise to a variety of diseases, including stroke, AS, thrombosis, hypertension, aneurysm, and so on. These diseases are directly related to inherent vessel disorders and result in numerous associated complications. In vitro vascular microsystems hold great promise as attractive and valuable tools for vascular pathology research in the future.

Tumor vasculature In the tumor microenvironment, tumor vasculature plays a crucial role in the regulation of transport of immune cells, physical barriers, tumor cell metastasis, and tumor hemodynamics[214,215]. Therefore, it is essential to incorporate blood vessels into in vitro cancer models as an integral component[216]. For instance, Cao et al. utilized 3D bioprinting to establish a vascularized microenvironment for MCF-7 breast cancer cells, facilitating the controlled study of vascular interactions during anticancer drug screening. This technical breakthrough directly addressed the limitations of conventional 2D systems by capturing the dynamic interaction between tumor cells and the surrounding vasculature[217]. Similarly, a novel bioprinting model of an artificial tumor, where endothelial and stromal cells autonomously organized into functional, perfusable vascular structures, has demonstrated that encapsulating multicellular tumor spheroids in 3D hydrogels can accurately represent diverse cancer stages[218]. The current research results have proved that the in vitro tumor vascular microsystems exhibit remarkable capabilities in replicating the complex tumor microenvironment, which is essential for investigating underlying mechanisms of tumor development and advancing the discovery of effective anti-tumor drugs.

Atherosclerosis (AS) AS is a chronic, multifactorial inflammatory disease characterized by the progressive narrowing of the arterial lumen due to lipid accumulation, fibrosis, and calcification[219]. In vitro models can appropriately reproduce the complexities and physiological relevance of vessel structures, they also provide a disease-related microenvironment with controlled mechanical stimulation[220]. The advancement of AS models at different stages can greatly contribute to elucidating biological mechanisms, pathology of disease progression, and risk factors, providing an effective platform for evaluating therapeutic drugs[221].

One major challenge in AS modeling is replicating the multi-layered architecture of the arterial wall and the complex hemodynamic forces that drive disease progression. Recently, Zheng et al. addressed this by developing a microfluidic model for early-stage AS that incorporated both cyclic stretch and fluid shear stress to which VECs were continuously exposed. By integrating these mechanical forces, the model effectively simulated the biomechanical and biochemical milieu of an artery, allowing for a comprehensive investigation into how AS-prone biochemical factors alter VEC behavior[222]. In an alternative coronary artery model, researchers focused on the effects of low and oscillatory shear stress (OSS) on VEC phenotype and subsequent activation of VSMCs. This system systematically analyzed the changes in endothelial phenotype in response to OSS, linking these alterations to increased atherogenic activity in VSMCs and exploring strategies to mitigate such effects[223]. Moreover, advances

in 3D bioprinting have facilitated the construction of stenosis models with precise, patient-specific geometries. Such systems not only replicate occlusive conditions but also allow for real-time monitoring of endothelial dysfunction and cell-cell interactions, which are crucial factors in understanding plaque development and progression[224].

Thrombosis Thrombosis is a pathological condition marked by abnormal clot formation due to excessive platelet activation and coagulation[225]. It poses significant challenges because it involves dynamic interactions among blood flow, vessel wall mechanics, and cellular responses. Traditional models have struggled to capture the full complexity of thrombogenesis. Recent advances in engineered in vitro microsystems, however, have begun to overcome these challenges by integrating structural, mechanical, and biochemical cues that closely mimic native vasculature. The future of bioprinted thrombosis models is anticipated to benefit from the integration of patient-specific data, real-time monitoring technologies, and sophisticated microfluidic platforms, ultimately promoting personalized medicine and targeted therapeutic interventions[226].

For instance, Zhang et al. employed a 3D bioprinting strategy to create a thrombosis-on-a-chip model that incorporated hollow channels lined with confluent VECs. This platform simulated the in vivo conditions by inducing thrombus formation via the perfusion of human whole blood. In addition, the encapsulation of fibroblasts within a GelMA matrix introduced elements of fibrosis remodeling. This design not only recapitulated the biological characteristics of thrombosis but also enabled the investigation of fibrosis-related pathological changes, which was an essential factor often missing in 2D models[227]. The incomplete understanding of pathogenic changes in the blood vessel wall and blood composition necessitates the use of in vitro models to elucidate these mechanisms and facilitate the development of safe and effective antithrombotic therapies[228]. In another innovative approach, Barrile et al. created a two-channel microengineered PDMS chip with a lower vascular microchannel and an adjacent anticoagulant port separated by a thin porous membrane. This design effectively replicated several key elements of thrombosis, including endothelial activation, platelet aggregation, fibrin clot formation, and thrombin anti-thrombin complex generation. By correlating in vitro findings with clinical data, this system demonstrated how precise microengineering can predict adverse drug reactions, thus offering a platform for evaluating antithrombotic therapies[229].

Aneurysms Aneurysms, characterized by localized dilations of blood vessels due to the structural deterioration of the vessel wall, present unique difficulties in in vitro modeling[230,231]. Aneurysm formation and rupture are driven by complex hemodynamic forces and intricate interactions between blood flow and vessel wall remodeling[232]. Conventional models have struggled to capture these dynamics, often reducing the phenomenon to static representations.

Recent engineering advances have provided innovative solutions. One approach leverages patient-specific data to inform computational fluid dynamics (CFD) simulations. For instance, Vivas et al. initially conducted CFD on the geometries of aneurysms in patients. They then simulated the site-specific flow dynamics within individual microfluidic channels, thereby demonstrating the feasibility of modeling distinct locations within separate microfluidic devices[233]. This strategy directly addressed the challenge of mimicking site-specific hemodynamics and their impact on vascular cell phenotype[234]. Alternatively, advanced 3D printing and molding techniques have enabled the direct fabrication of patient-specific aneurysm models. Kaneko et al. developed a geometrically realistic intracranial IA model with live endothelial cells. By combining CFD analysis with 3D-printed patient-specific geometries, this approach allowed for the comprehensive examination of how complex flow patterns affected endothelial morphology and triggered pathological remodeling[235]. Similarly, Tian et al. designed a transparent blood vessel-on-a-chip compatible with magnetic resonance imaging, which facilitated the visualization of hemodynamic variations throughout different stages of IA growth[236]. The integration of imaging and microfluidics strategies provided a powerful tool for early detection and continuous monitoring of aneurysmal progression.

However, because the initiation and growth of an aneurysm is a gradual process, the current in vitro aneurysm models either simply extract hemodynamics data or only simulate the fixed-size aneurysm. A physiologically relevant in vitro aneurysm model is currently unavailable, hindering the ability to observe and analyze the dynamic progression of this disease in real-time.

Reconstruction of vascularized tissues and organs

In vitro tissue and organ models are used to reproduce complex and intricate physiological structures, thereby promoting pathophysiological modeling and advancements in drug development. The introduction of vascular systems or vascular interfaces is especially critical for in vitro tissue and organ models, as they enable realistic reconstruction of certain reactions and mechanisms that can only be faithfully replicated with endothelial-lined vasculature.

Brain

The brain is characterized by its unique vascular network, particularly the BBB, which regulates molecular transport while protecting the central nervous system from toxins and pathogens. Traditional models struggle to replicate the physiological shear stress, selective permeability, and cellular interactions of the BBB[237]. Advances in microfluidics and biomaterials have led to the development of brain-on-chip platforms that incorporate crucial components, primarily the BBB and neurovascular units[238,239]. However, challenges remain in achieving physiologically relevant channel diameters and flow conditions, as well as extracellular matrix compositions that replicate healthy brain tissue properties[240]. Recent microfluidic BBB models have recreated key features of the neurovascular environment. For example, Kim et al. established a human neurovascular-unit-on-a-chip incorporating human neural stem cells, microvascular endothelial cells, and pericytes, effectively replicating BBB function. This system provided a powerful tool for studying microbial penetration, infectious brain disorders, and target drugs[241]. Moreover, integrating vascularized microsystems with stem cell technologies holds promise for modeling neurodegenerative diseases such as Alzheimer's, Parkinson's, amyotrophic lateral sclerosis, and Huntington's disease, offering a platform for drug screening and mechanistic studies[242,243].

Heart

The heart, as one of the most densely vascularized organs, requires an intricate capillary network to sustain its high metabolic demands. The animal models and 2D cell cultures fail to replicate the biomechanical and electrophysiological environment necessary for cardiac maturation and function[244]. The integration of perfusion-based microsystems with cardiomyocyte cultures has enhanced oxygen and nutrient delivery while enabling real-time functional assessment[245]. A major technical milestone in vascularized cardiac models was the development of 3D engineered heart tissues incorporating VECs. Early studies by Caspi et al. demonstrated that co-culturing cardiomyocytes with VECs enhanced cell proliferation and differentiation, improving tissue organization and contractile function[246].

Lung

The intricate structure of the lung, particularly the alveolar-capillary interface, poses significant challenges for conventional in vitro modeling. Recent innovations have led to the development of 3D microfluidic culture systems that co-culture pulmonary epithelial cells and VECs to reconstruct the alveolar-capillary barrier[247]. A key breakthrough was the design of a sandwich structure featuring a thin, flexible membrane that separates an air-filled alveolar channel from a liquid-filled vascular channel. Stretching this membrane simulated the cyclic mechanical deformations characteristic of breathing, thereby more accurately reproducing lung physiology[248–250].

The first reported model with a relatively complete physiological function of breathing lung was developed by Huh et al., who created a multifunctional microdevice that incorporated a tissue-tissue interface of epithelial cells and VECs along with ECM, to model the essential structural, mechanical, and functional properties of the alveolar-capillary interface. This platform not only replicated the responses to cytokine and pathogen-induced inflammation but also facilitated the establishment of a pulmonary edema model for drug toxicity screening[248,251]. Similarly, Bai et al. subjected an engineered alveolar-capillary interface to cyclic mechanical deformations that simulate the natural breathing motions and respiratory rate of humans. It was achieved by applying cyclic suction to hollow side chambers within a flexible PDMS device, which revealed how breathing motions inhibited viral replication through a unique mechano-chemical regulatory mechanism[250]. These technical developments directly address the challenge of modeling dynamic, vascularized lung tissue in vitro.

Liver

The liver is the largest internal organ in the human body, with an excellent ability for regeneration, metabolism, and detoxification[252]. A proper supply and drainage of blood flow are crucial for the functioning of the liver, and understanding blood flow and its regulation would be a key to clarifying liver physiology and pathology. To effectively model and simulate the complex functions in vitro, it is crucial to construct an endothelial-barrier structure of blood vessels, with particular emphasis on employing a co-culture and flow loading system to enhance hepatic functionalities[253,254].

Mi et al. successfully replicated the liver vascular structure by using microfluidic chips to create a liver sinusoid model. By injecting collagen-containing hepatocytes and endothelial cells into separate chambers, they achieved a layered co-culture that reproduced the in vivo-like endothelial barrier and nutrient flow that were essential for liver functionality. This approach not only reconstituted physiological structures but also enabled the evaluation of hepatotoxicity through controlled drug dosing[255]. Jang et al. further advanced liver microsystems by developing species-specific liver chips. In these devices, hepatic cells from rats, dogs, or humans were cultured in an ECM-rich upper parenchymal channel, while species-specific liver sinusoidal endothelial cells and non-parenchymal cells were maintained in the lower vascular channel. This configuration preserved native liver

cytoarchitecture for extended periods and enabled dynamic assessment of drug-metabolizing capacity and toxicity. Their research demonstrated that this species-specific in vitro liver model held promising potential for accurately predicting outcomes across various species. Moreover, it can serve as a valuable tool for assessing safety and risk, particularly determining the relevance of drug-induced liver toxicities observed in animal models to humans[256].

Kidney

The ability of the kidney to filter blood, reabsorb essential nutrients, and maintain homeostasis of salt and water is governed by its complex 3D vascular architecture and the interplay among diverse cell types, including VECs, mesangial cells, podocytes, etc. Engineering in vitro 3D kidney microsystems addresses these challenges by integrating advanced microfluidic designs and co-culture techniques that mimic native kidney structures and functions[257]. The establishment of in vitro 3D kidney models with diverse kidney cell types within a microfluidic system allows for the replication of the main components of the kidney[258]. Vedula et al. successfully engineered a microfluidic renal PT model that accurately mimicked physiological architecture and barrier function, effectively replicating the process of solute reabsorption into the peritubular capillaries through active transport. Co-culturing epithelial cells and VECs in this model significantly enhanced both the formation and activity of the epithelial layer, leading to improved sodium-coupled glucose transport performance[259]. Another innovative research involves recreating the glomerular-capillary wall, which is critical for molecular filtration. In this system, podocytes derived from human iPSCs were co-cultured with primary human glomerular endothelial cells on opposite sides of a flexible PDMS membrane. This configuration replicates both the urinary and capillary compartments of the glomerulus. The integration of side chambers to simulate the dynamic mechanical strain induced by the cyclic pulsations of renal blood flow further refines the physiological relevance of the model[260]. This design overcomes traditional limitations by closely reproducing the tissue-tissue interface and mechanical forces essential for kidney function, providing a robust platform for studying renal toxicity and disease mechanisms.

Gut

The gut, as the largest organ for digestion and absorption, presents unique challenges for in vitro modeling due to its complex architecture, diverse cell populations, and dynamic interactions with a resident microbiome[261]. The vascular channel may be a conserved feature amongst in vitro gut models, as it accurately replicates the barrier properties of native vascular tissue, which is indispensable to studying the absorption and transport of small molecules[262]. In addition, Gut microbes play a role in regulating angiogenesis within the tumor microenvironment and also affect the effectiveness of immune checkpoint inhibitors[263]. Most in vitro gut models are designed as two microchannels, separated by a porous and flexible membrane, to simulate the intestinal lumen and the flowing vascular lumen. The membrane allows for the transport of soluble molecules between these microchannels. For example, Kasendra et al. developed an in vitro intestine model that applied physiologically relevant flow in both channels, successfully replicating the barrier properties and absorption functions of native intestinal tissue. This design directly addressed the challenge of replicating nutrient uptake and drug absorption under dynamic circumstances[264]. Incorporating a layer of VECs close to the intestinal epithelium has been proven essential for replicating capillary blood vessels. Such configurations allowed for the study of intestinal radiation injury and the evaluation of potential countermeasure drugs. This approach highlighted the importance of accurately modeling the vascular component to understand how systemic factors influence gut function[265].

Skin

The skin serves as the largest body organ and functions as both a physical and immunological barrier. Its complex multilayer structure is underpinned by a rich network of blood vessels that support nutrient delivery, immune responses, and tissue regeneration[266]. Currently available in vitro skin models typically only recapitulate either the single epidermal layer or both epidermis and dermis layers. However, it is essential to incorporate vascularization into these models to ensure adequate oxygen and nutrient supply, contribute to immune responses within the skin tissue, as well as regulate the microenvironment of the skin for effective regeneration[267]. The in vitro vascularized skin models offer a more comprehensive representation of human skin complexity. Mori et al. fabricated a skin-equivalent model with perfusable vascular channels to address the problem of the oxygen and nutrient supply. In this system, VECs were seeded into the dermal layer and successfully induced vascularization under controlled perfusion conditions, and established distinct dermal/epidermal layers with effective barrier function. This development not only improved the physiological relevance of the model but also enabled quantitative studies of drug absorption via the vascular network[268]. To further enhance the construction of an accurate 3D skin model, Kim et al. employed an innovative cell-printing platform to construct a fully developed, perfusable, vascularized 3D full-thickness skin model. This system integrates vascularized dermal and hypodermal compartments, facilitating

physiological crosstalk during keratinocyte differentiation. It more accurately captured the intricate nature of native human skin, thereby providing a more effective and dependable in vitro platform for modeling skin disease and exploring pathological mechanisms[269].

Bone

Bone has a rich network of blood vessels, which plays a crucial role not only in nutrient and waste exchange but also in regulating bone formation and healing[270]. It is necessary to develop the vascularized bone models to accurately reconstruct the natural physiological and pathological environment of bones in vitro. By incorporating functional microvascular networks, such models can enhance mass transport, support cell viability, and mimic key cellular interactions, thus providing a more physiologically relevant platform for studying bone biology and disease[271]. Chou et al. developed a vascularized human bone marrow-on-a-chip that combined a fibrin gel-based co-culture of CD34+ cells and stromal cells with an adjacent vascular channel lined by VECs. The vascular channel can provide a continuous supply of fresh medium and remove accumulated soluble waste products, thereby maintaining the system in an optimized equilibrium state. CD34+ cells cultured in this chip exhibited hematopoietic dysfunctions similar to clinical observations. Consequently, the utilization of this model had the potential to enhance drug development, fundamental research, and translational investigations pertaining to hematopoietic diseases[272]. Additionally, in vitro vascularized bone models are extensively employed within the field of cancer treatment and prevention[273].

Drug development

Developing new drugs is a slow-paced and cost-intensive endeavor, characterized by complex scientific requirements and economically challenging. A new drug from preclinical studies to the market would take over a decade and is estimated to cost 2.5 billion dollars. Unfortunately, less than 10% of the drug candidates can successfully progress into human clinical trial phases[274]. A major contributor to clinical failures is the inherent species-specific variations between humans and animals used in these trials, leading to inaccurate predictions of some key pharmacokinetic (PK) and pharmacodynamic (PD) parameters such as drug concentration, toxicity, and efficacy. In particular, the endothelium makes a major contribution to drug toxicity, absorption, distribution, metabolism, and excretion processes, since drugs are transported within the vascular lumen and traverse the endothelial barrier to reach parenchymal tissue in vivo[163,275]. Engineered in vitro vascular microsystems have emerged as a powerful solution to these challenges by recapitulating the physiological complexity of human blood flow and vascular interactions.

Traditional static cultures lack the dynamic biomechanical cues provided by blood flow, which are essential for accurately assessing drug responses. Nashimoto et al. developed an engineered tumor vascular network to recapitulate the in vivo tumor microenvironment, supplying nutrition, oxygen, and drug delivery through the vascular network to the tumor tissue. Compared to the drug response in static conditions, the administration of an anticancer drug did not result in any dose-dependent reduction in tumor cells in perfused conditions. This finding highlighted the significance of blood flow in accurately assessing tumor activities within the drug screening platform[276].

PK/PD parameters analyses require multi-organ systems linked by vascular perfusion to replicate the natural sequential drug reactions occurring in multiple body organs. For instance, Herland et al. developed a first-pass model that effectively simulates the processes of drug absorption, metabolism, and excretion in humans. This was achieved by interconnecting organ-chip models of the gut, liver, and kidneys through vascular endothelium-lined channels[277]. Subsequently, these physiologically based PK models were constructed to predict quantitative in vivo PK parameters for nicotine and cisplatin, which exhibited a high degree of consistency with those obtained from human clinical studies. those obtained in human clinical studies. Additionally, an organ-on-a-chip platform integrating an intestine-blood vessel-liver-kidney system has been used to systematically analyze the absorption, metabolism, and toxicity of ginsenoside CK, with findings consistent with conventional traditional PK testing methods[278]. These results suggest that these models would be valuable tools for studying and predicting drug PD/PK.

Personalized medicine

Personalization is defined as the customization of medical treatment to suit the unique characteristics of individual patients. More specifically, it involves categorizing individuals into distinct populations based on their unique or disproportionate susceptibility to specific diseases or commonly used treatments. In vitro vascular microsystems are emerging as essential tools in this endeavor because they can combining patient-derived cells to model patient-specific characteristics and study individual differences, providing more precise clinical diagnosis and individual drug screening eventually, thus identifying the most effective individualized therapy[279,280].

In clinical practice, patients are often prescribed drugs from similar therapeutic categories, according to their disease types and clinical symptoms. However, the efficacy, tolerability, and toxicity of these drugs may be highly

variable among different populations, such as age, sex, race, region, and lifestyle[281]. For instance, Petrosyan et al. developed an in vitro glomerular filtration barrier model using human podocytes and glomerular endothelial cells derived from a patient with Alport syndrome. By generating multiple cell lines from patients with distinct genetic mutations, this approach not only recapitulated the pathology of the disease but also allowed for the observation of disease heterogeneity within a dynamic system[282]. iPSCs have the potential to be leveraged in the practice of precision medicine that faithful proxies for the tissues in the living human being, especially when the patient-derived primary cells are challenging to obtain[283]. Recently, the construction of iPSC-derived BBB organ-on-chip has been achieved, and the system has been proven to detect functional differences between healthy individuals and patients with neurological disorders[284]. The application of 3D bioprinting in the development of vascular microsystems holds great promise for personalized medicine and has emerged as one of the most exciting advancements in healthcare. These models accurately replicate both normal anatomical structures and pathological conditions specific to individual patients, using imaging datasets[285].

Commercialization of in vitro vascular microsystems

The majority of the in vitro vascular microsystems discussed in this review have been primarily developed by researchers and are currently limited to laboratory use. However, there is a growing number of emerging companies that are gradually transitioning towards commercialization and making these models available on the market. Most commercially available in vitro vascular microsystems, predominantly developed by companies, are organ-on-chip systems that incorporate vasculature. The establishment of vasculature within both single-organ chips and multi-organ chips is crucial for the development of in vitro tissue and organ models with complete functionality. Here, we introduce several companies dedicated to advancing vascularized organ-on-chip technology and relevant products.

Mimetas, Inc., established in Leiden in 2013, aims to make significant contributions to innovative treatments by utilizing versatile technology platforms to identify and study human disease through reliable and screenable assays. Their OrganoPlate™ system excelled in modeling angiogenesis and barrier function with high imaging quality and automation compatibility (Fig. 8a)[286]. It is well suited for studies requiring detailed assessment of endothelial dynamics, such as drug-induced toxicity or the immune cell interaction within vascular barriers. It has also been applied to reconstruct several physiological and pathological processes of blood vessels in vitro, such as immune cell interaction[287,288], defective angiogenesis in systemic sclerosis[289], and ischemic stroke[290].

Hesperos Inc. was founded in 2015 by Drs. Shuler and Hickman with the aim of expediting the process of drug

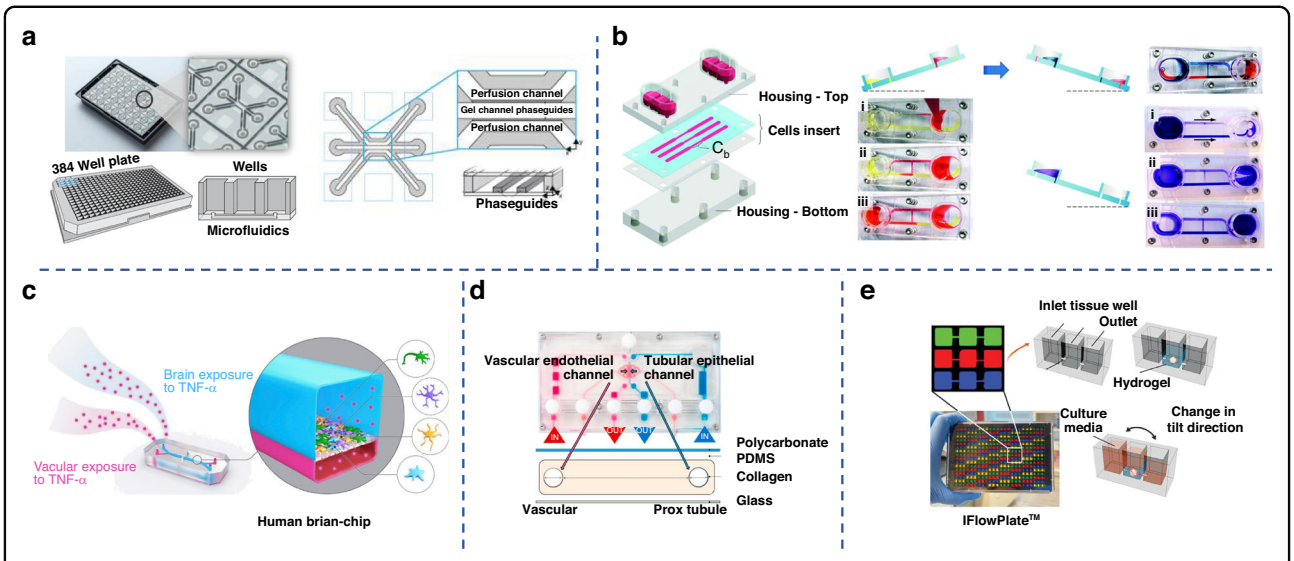

Fig. 8 Commercial vascularized organ-on-chip and relevant products. a The OrganoPlate® platform based on a 384-well plate. Every device consists of three channels: one 'gel' channel for gel patterning, and two adjacent channels[286]. Copyright 2018, Springer Nature. **b** Schematic exploded view of the BiChip, and the flow direction was visualized with dyes[292]. Copyright 2018, Royal Society of Chemistry. **c** Schematic illustration of the Brain-Chip, a two-channel microengineered chip including multiple types of brain cells[293]. Copyright 2022, Cell Press. **d** Nortis dual-channel platform used for the establishment of VPT-MPS[295]. Copyright 2022, American Chemical Society. **e** An IFlowPlate model filled with different colored dyes to depict the 128-independent units and illustration of and experimental set up for vascularization of organoids cultured in the device[296]. Copyright 2017, Wiley-VCH

discovery through the utilization of the Human-on-a-Chip® technology. As early as 2011, the square microfluidic vascular channels and the semi-circular channels were studied and described[291]. The "UniChip" fluid network design, developed by Wang et al., converted reciprocating flow input into one-directional perfusion in the desired channel by supporting channels and passive valves. It allowed for the integration of blood vessels and other tissues that were sensitive to shear stress, such as the lungs and kidneys, into pumpless organ-on-chips with long-term recirculating unidirectional perfusion (Fig. 8b)[292]. Focusing on replicating human diseases in vitro, their platforms are ideal for multi-organ integration in disease modeling and drug testing.

Emulate Inc.'s founding team was the pioneers of the organs-on-chip technology, which was developed at the Wyss Institute located at Harvard University. Their products typically encompass miniature hollow channels lined with an array of living human cells, resembling the size of an AA battery, aiming to replicate the physiological conditions and mechanical forces. They successfully reconstituted the cerebrovascular interface to model the BBB within a chip, aiming to gain mechanistic insights into cellular interactions and BBB functionality during neuroinflammation. This cutting-edge platform also enables disease-specific modeling capabilities (Fig. 8c)[293].

Nortis Inc. is a biotech company that has been deeply involved in the field of organ-on-chip for over a decade. The unique ParVivo™ platform they developed can create tubular structures in the biological matrix, as well as real 3D tissue, which is suitable for cavity tissue structures with tight perfusion. In the early ParVivo™ platform, 3D engineered human microvasculature has been used to create models of angiogenesis[294]. In addition, this platform has been used to develop several vascularized kidney models for investigations of mechanisms of drug-induced nephrotoxicity. Alenka Chapron et al. used Nortis vascularized human PT model in a dual-channel microphysiological system to replicate the functions of vascular endothelial cells and PT epithelial cells. It contributed to assessing tubular secretion and drug-induced kidney injury (Fig. 8d)[295].

As an emerging company, OrganoBiotech, Inc. has created and marketed a tissue culture platform called IFlowPlate™, utilized for cultivating organoids with integrated vascular networks. Its 384-well "open-well" design eliminated the need for external pumps while enabling the formation of blood vessels around various organoids (Fig. 8e). The vascularized colon organoids[296], vascularized liver spheroids[297], and vascularized crypt-patterned colon model[298] have been developed using the IFlowPlate technology.

Challenges and prospects

The physiological in vitro vascular microsystems have enormous potential to replace animal testing and 2D cell culture as the standard model, expedite clinical trials, and open new avenues for personalized medicine. Remarkable advancements have been achieved in the development of in vitro vascular research platforms over the past few decades that have addressed many types of questions in human biology and medicine that cannot be answered using traditional experimental models. However, realizing vascular microsystems that faithfully replicate the in vivo environment requires overcoming some complicated challenges. At the same time, recent advances in materials science, microfabrication, and bioengineering are paving the way for evolution.

The choice of biomaterials and fabrication methods is critical for constructing in vitro vascular microsystems that support long-term cell viability and accurate physiological function. Commonly used materials like PDMS offer excellent optical clarity and ease of fabrication via soft lithography, yet they often suffer from issues such as limited cyto-compatibility, absorption of small molecules, and incompatibility with advanced 3D bioprinting techniques. Innovations in biomaterials are expected to yield next-generation bioinks and polymers that provide a balance between mechanical stability, biocompatibility, and dynamic responsiveness. Emerging strategies include the development of stimuli-responsive polymers that change properties in response to environmental cues to construct an in vitro microenvironment that more suitable for the formation of the vascular system[299], such as photo-caged RGD modified hydrogels induced a light-guided migration of angiogenesis[300]. Advances in multi-material 3D printing are also anticipated to allow for the precise deposition of heterogeneous materials, enabling the fabrication of vascular networks with tunable properties that closely reflect the complexity of native vascularized tissues/organs[301]. For example, the PDMS hybrid inks have been developed to additively manufacture high-precision architectures with a feature size of ≈100 μm[302,303].

Traditional microfabrication techniques, such as lithography and templating, excel at generating high-resolution, uniform microchannels but often fall short in replicating the 3D architecture and dynamic functionality of natural blood vessels. The static nature of these constructs limits their ability to mimic the mechanical forces that are crucial for vascular function. Additive manufacturing has extended beyond the capabilities of traditional 3D bioprinting, now incorporating more advanced technologies such as 4D, 5D, and 6D bioprinting. While 3D and 4D bioprinting of vascular structures involves creating static, passive blood vessels that do not actively interact with the environment, these models can be used

to study basic vascular functions or for drug testing under controlled conditions. However, they lack the ability to adapt or dynamically respond to physiological changes. In contrast, 5D and 6D bioprinting technologies can create more advanced intelligent in vitro vascular microsystems that can simulate real-time interactions within the vascular system, such as responses to blood flow, pressure, and environmental changes[83,84]. The integration of real-time sensing and feedback systems within the fabrication process may further enhance the physiological relevance of these models[304]. Moreover, the emergence of robotic fluid coupling systems fulfills the needs of an automated experimental system and realizes multiple vascularized organ chips for PK and PD analysis[305].

Combining iPSCs with in vitro vascular microsystems enhances cellular fidelity, thereby establishing an effective tool for precise drug administration and personalized medicine. The in vitro vascular microsystems have witnessed significant advancements, *evolving* from endothelial cell monoculture to the co-culturing of multiple types of vascular cells. A more intricate microarchitecture can complicate nutrient delivery, waste removal, and the management of biochemical gradients, potentially leading to the failure of cell models, an increase in experimental costs, and reduced overall efficiency[306]. Moreover, given the inherent pluripotency of iPSCs, another concern arises: whether these cells maintain their pluripotent state when cultured on complex chips or, if directed into specific lineages, whether they successfully retain their target cell characteristics over the long term. To address these issues, enhanced co-culture systems that promote dynamic crosstalk between vascular and organ-specific cells must be developed. Integrated sensors for real-time monitoring of key biochemical and biomechanical parameters (such as oxygen levels, nutrient gradients, and shear stress) offer promise in providing continuous feedback, thereby enabling the dynamic adjustment of culture conditions to preserve iPSC viability and phenotype[304].

Personalization constitutes a necessary route for in vitro vascular systems and is expected to revolutionize biomedical research by bridging high-throughput methodologies with individualized approaches. These systems uniquely enable the cultivation of patient-derived endothelial and stromal cells within physiologically relevant 3D microenvironments, replicating individualized vascular dynamics such as shear stress patterns, barrier permeability, and organ-specific interactions[307]. By integrating vascularized microphysiological platforms with automated screening technologies, researchers can rapidly identify optimal therapeutics tailored to a patient's genetic profile or disease phenotype[308]. For instance, predicting antiangiogenic drug efficacy or resolving vascular dysfunction in diabetic retinopathy[309,310]. This synergy not only accelerates drug discovery but also ensures therapies can be finely adjusted according to individual vascular biology, ranging from endothelial-specific protein expression to vascular disease development. As these systems evolve, they will unlock accurate in vitro to in vivo prediction for personalized medicine, offering unprecedented precision in the treatment of vascular diseases.

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Author details
[1]West China School of Basic Medical Sciences & Forensic Medicine, Sichuan University, Chengdu, China. [2]Jinfeng Laboratory, Chongqing, China. [3]Tianfu Jincheng Laboratory, Chengdu, China. [4]Department of Chemical Engineering, Imperial College London, South Kensington, London, UK

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