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Engineered Vasculature for Organ-on-a-Chip Systems

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ABSTRACT

Organ-on-a-chip technology, a promising three-dimensional (3D) dynamic culture method, ensures accurate and efficient cell culture and has great potential for replacing animal models in preclinical testing. The circulatory system, the most abundant organ in the human body, plays a crucial role in oxygen exchange and mass transfer, which is the determining factor for the survival of tissues and organs. Thus, it is essential to integrate the circulatory system into an organ-on-a-chip to recreate tissue and organ microenvironments and physiological functions. This review discusses the synergy between the vasculature and the emerging organ-on-a-chip technology, which offers even better possibilities of duplicating physiology and disease characteristics. In addition, we review the different steps of a vascularized organ-on-a-chip fabrication process, including structure fabrication and tissue construction using different biofabrication strategies. Finally, we outline the applicability of this technology in the fascinating and fast-developing field of organ and tumor culture.

1. Introduction

Researchers have made countless advances in developing and testing drugs to combat deadly diseases. However, the completion and approval of a drug takes approximately 12 years, which can sometimes cost millions of lives [1]. Additionally, using two-dimensional (2D) cell culture models and animal models during the preclinical testing phase of new drugs has led to a low approval rate during the last decade [2,3]. The failure of 2D cell cultures to predict the effects of drugs accurately is primarily due to the lack of similarity between these cultures and a three-dimensional (3D) microenvironment and its static culture conditions, and animal models are comparably expensive and anatomically different from the human body. Therefore, new *in vitro* cell culture methods have recently been developed to achieve adequate and efficient tests [4,5].

On the one hand, research on organ-on-a-chip technology has pushed cell culture methods toward greater accuracy. Generally, an organ-on-a-chip can be defined as a microfluidic device containing organ-specific cells and simulating organ-level functions. It is also a useful tool that can be easily controlled, analyzed, and most importantly, can mimic complex tissues in a miniaturized volume. This new approach, which arguably has more advantages than the traditional 2D monolayer static cell culture method, has proved to be a better alternative to animal models in terms of its capacity to culture human tissues, cost, and ethical and public concerns [6]. The feasibility of the organ-on-a-chip device is another major incentive that has pushed researchers to apply it to mimic several human organs such as the heart [7–10], lung [11–13], liver [14–16], skin [17–19], brain [20–22], and kidneys [23,24].

On the other hand, mimicking an *in vivo* organ requires detailed knowledge of its functions. The most abundant organ in the human body is the vascular system, a circulatory system of vessels carrying oxygen and nutrients to other body systems, including the respiratory system, the digestive system, the kidneys, and the urinary system. Accordingly, the vasculature plays an essential and critical role in maintaining the body in a steady-state and ensuring optimal organ function. Thus, integrating a system of nutrient and oxygen supply, in other words, the vasculature, into organs-on-a-chip is necessary to recreate the microenvironment and physiological functions of the organ (Fig. 1).

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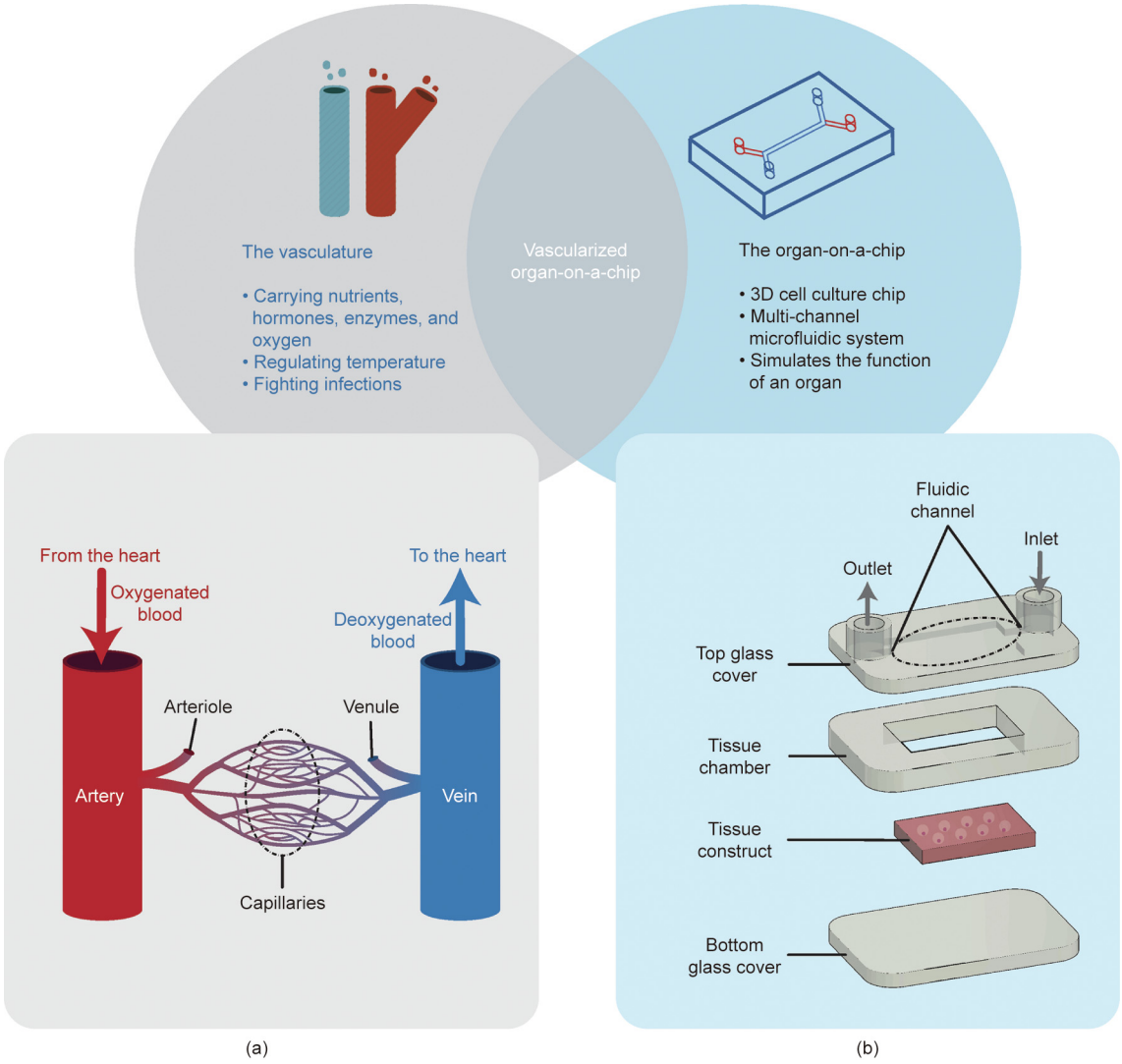


Fig. 1. Main functions of the vasculature and an organ-on-a-chip, and their intersection on a vascularized organ-on-a-chip. (a) Schematic of a circulatory system including capillaries and blood flow; (b) basic structure and compounds of an organ-on-a-chip.

In this review, we focus on the construction of a vascularized organ-on-a-chip device. First, the vasculature and organ-on-a-chip fabrication process is described with particular attention to two critical steps: chip structure manufacture and tissue construction. The importance of applying vasculature on several organ-on-a-chip and tumor-on-a-chip devices will be demonstrated by describing various recent and highly accurate models, which would help identify a new, promising avenue for drug screening.

2. Fabrication of the vascularized organ-on-a-chip

Organs-on-a-chip and vascularized organs-on-a-chip have been fabricated using several innovative methods [25]. These approaches substantially vary based on the targeted characteristics to be achieved in the model. Generally, the fabrication of an organ-on-a-chip device is completed in five steps (Fig. 2). The first step is the designing of the organ-on-a-chip platform and modeling it in 3D. The next step is to manufacture the structure of the device using either lithography-based methods or other methods that allow the use of a compatible material. After manufacturing the device, tissue construction is generally achieved using a microfluidic or bioprinting strategy. The organ-on-a-chip is typically composed of several layers because of the complexity of the tissue and the structure of the device; moreover, the microfabrication method has limitations in the second step of the process, which requires a bonding step to connect all the parts and form the chip. The device is then connected to a fluid circulatory system that carries nutrients or drugs, allowing precise control of the entire system. In some relevant studies [26–28], fabrication was achieved in only three steps by completing the device and tissue fabrication in a single step using a bioprinting technique that does not require bonding. Finally, fabrication of the platform structure and bioprinting of the tissues, the most challenging and vital steps, especially in the creation of vascularized tissues for organs-on-chips, are achieved; we have described them in detail.

2.1. Designing the organ-on-a-chip platform

The first is a prefabrication step, which consists of designing the device and modeling it in 3D, including the cover, tissue chambers, tissue constructs, and fluidic channels, using computer-aided design. This is subsequently transformed into a standard triangle language file when a 3D printing strategy is chosen. Notably, for the construction of a vascularized organ-on-a-chip, this step requires additional modeling of the vasculature [29]. Therefore, a

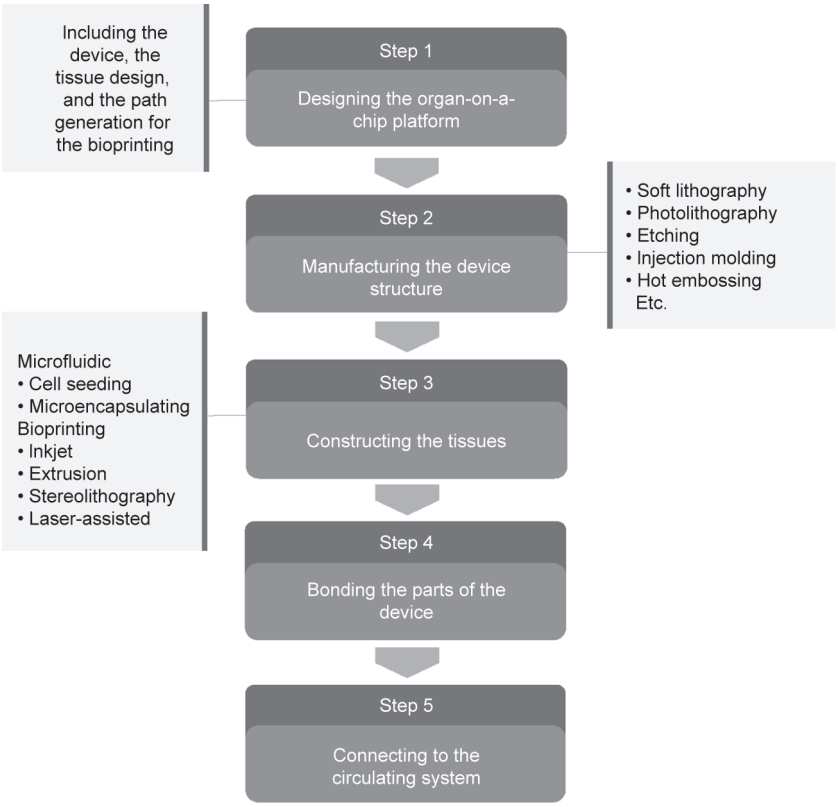


Fig. 2. The fabrication process of an organ-on-a-chip device.

prelaminar study to choose the appropriate vasculature model is of utmost importance. The vasculature model is chosen based on the targets of the project, such as geometrical complexity or high functional efficiency of the vasculature. Because the current advances in vasculature models have still not led to a complete model, the choice of a suitable model brings into play the advantages and disadvantages of different biofabrication methods, which will be described later in this paper.

2.2. Manufacturing the device structure

Currently, microfabrication methods are intensely being developed using a wide variety of techniques described in the literature. Therefore, the following section will focus on newly developed methods, the most commonly used lithography-based methods, (including soft-lithography and photolithography methods), and other non-lithographic methods.

2.2.1. Soft-lithography method

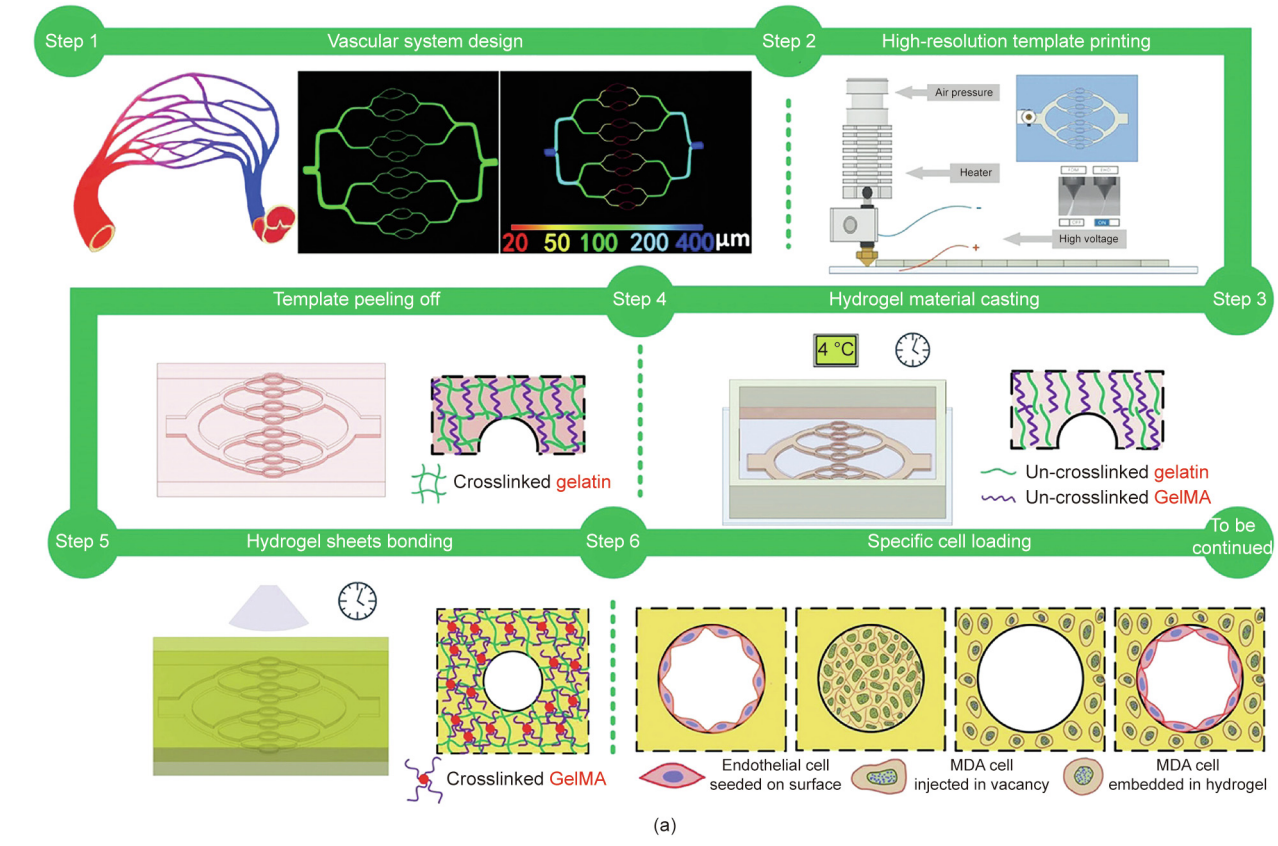
Soft lithography is a method widely used to replicate a structure using a prefabricated mold [30]. The "soft" in the name comes from the elastomeric nature of the material. Polydimethylsiloxane (PDMS) is generally considered the most suitable material for this method. The main advantages of soft lithography are its high micro-size precision and cost-effectiveness. It involves replica molding (REM), microcontact printing, micro-molding in micro-capillaries, micro-transfer molding, and solvent-assisted micro-molding.

In general, REM is the most popular soft-lithography method. The REM method starts with constructing a master mold that will be used for a secondary cross-linkable material. After this material is molded, the mold is detached, followed by replication in the reverse mode [31]. The same process was used by Zheng et al. [32] to create micro-vessels on a chip, enabling the study of angiogenic activities and the thrombotic nature of endothelialized microfluidic vessels. Additionally, Miali et al. [33] proved the versatility of this method, that is, the REM process was used to create authentically complex microvasculature networks inspired by a *Hedera elix* leaf. Freshly collected leaves were taped to obtain a negative print in a PDMS mold. Next, the replica was used to create a SU8-5 template and achieve a sandwiched system mimicking the complex geometrical and biological properties of the human vasculature.

Similarly, Nie et al. [34] considered the complex structure of a real *in vivo* vascular network as a target to mimic. They chose to proceed at three different levels (Fig. 3(a)) [34]. First, to manufacture a high-resolution template, 3D printing was integrated into the process. A classic casting, peeling, and bonding process was used, but interestingly was combined with a dual crosslinking strategy to obtain a hollowed platform allowing the final step, specific cell loading.

2.2.2. Photolithography method

Photolithography (also known as optical lithography or ultraviolet (UV) lithography) is based on transferring parts from a mask to a bulk or thin film [35–37]. This technology enables high precision within a range of a few nanometers and allows the fabrication of comparatively complex structures, making it an accurate and powerful tool to construct vasculature-like microchannels. However, this is a time-consuming and relatively expensive method. In a recent study, Fenech et al. [38] proposed a new photolithography-based technique to fabricate a vasculature that was geometrically close to real natural vasculatures. The process was based on using backside illumination and an optical diffuser to create a SU-8 photoresist mold, which has controllable rounded sections and direct proportionality between height and width (Fig. 3(b)) [38].



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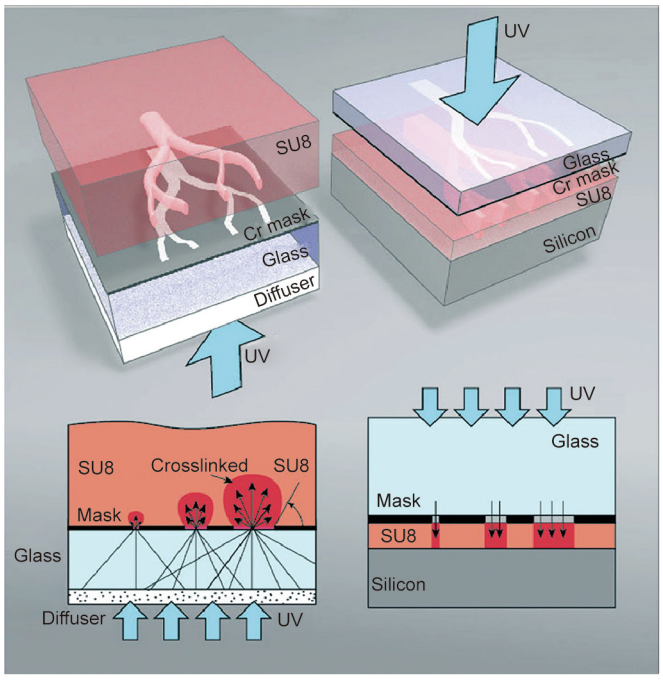

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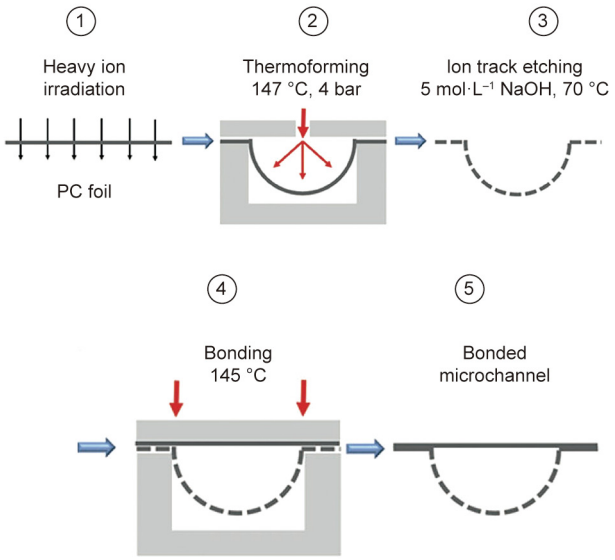

(c)

Fig. 3. Microfabrication methods of vascularized platforms. (a) Soft-lithography method: the fabrication process of a multiscale vascular chip using 3D printing and dual-crosslinking techniques. GelMA: gelatin-methacryloyl. (b) Photolithography method: a new photolithography technique is shown on the left and on the right is the standard photolithography technique. (c) Non-lithographic method: the SMART process was used to fabricate a microchannel. PC: polycarbonate. 1 bar = 10^5 Pa. (a) Reproduced from Ref. [34] with permission of the Royal Society of Chemistry, ©2020; (b) reproduced from Ref. [38] with permission of the Royal Society of Chemistry, ©2019; (c) reproduced from Ref. [39] with permission of Wiley-VCH Verlag GmbH and Company KGaA, ©2018.

2.2.3. Non-lithographic method

Although lithography-based methods have several limitations, researchers have never stopped improving them and proposing solutions to overcome these problems. One of the most frequently encountered limitations in the microfabrication of an *in vitro* vasculature is recreating a rounded cross-section, which motivated

Kappings et al. [39] to develop a new technology called vasQchip. Generally, to simplify an element of a machine function, it is advisable to look for its symmetries. Thus, the same reasoning could be applied to the tubular geometry of the real vasculature to create a simplified model. In the same study, the research team constructed a semicircular scaffold with a porous microchannel. The fabrication process of the channels was called substrate modification and replication by thermoforming (SMART) technology. It begins with irradiation of a polycarbonate film with heavy ions and then using the micro-thermoforming process to create a semicircular form to bond the microchannel (Fig. 3(c)) [39]. However, no recent studies have demonstrated the ability to fabricate a multiscale and ubiquitous vasculature structure using the SMART technology.

2.3. Constructing the vascularized tissues

Three models of constructing vascularized tissues have been proposed by several research teams during the last decade, including the endothelial barrier-based model and the vascular formation-based model (the angiogenesis and vasculogenesis models) (Fig. 4) [40,41]. The endothelial barrier model involves creating a 3D structure by patterning endothelial cells (ECs) on an organ-on-a-chip device wall [42–47]. Generally, this model is selected for its feasibility and controllability, although it is not reliable for mimicking angiogenesis and vasculogenesis. The vasculogenesis model is based on the differentiation of ECs with *de novo* vascular network formation. The angiogenesis model is constructed by growing and sprouting new capillaries from existing blood vessels [48–55]. More importantly, synergy between the endothelial barrier-based model and the vascular formation-based model was achieved by Wang et al. [54] to create a device allowing a tight connection between the artery/vein and the capillary networks, which could also be an efficient tool for interconnecting several organ tissues and to create a body-on-a-chip platform.

After choosing the vasculature model, one must select a fabrication strategy for the microvasculature and organ tissues. Two main strategies exist: the microfluidic strategy, which uses the micromechanical and biochemical behavior of targeted cells under well-defined microfluidic conditions to control their positioning in the device; and the bioprinting strategy, which is based on direct cell or tissue deposition.

2.3.1. Microfluidic strategy

Integrating ECs in an organ-on-a-chip device is fundamental to ensuring vascular–organ interactions. Microfluidic physics is the oldest most widely used method for controlling cells in microphysiological systems. The microfluidic strategy involves applying microfluidic pressure on perfused cells to encapsulate them under certain structural and functional conditions. There are two main microfluidic methods, namely the wall-trapping method and the microencapsulation method (also known as the self-assembly method).

2.3.1.1. Wall-trapping method. Recreating vasculature in an organ-on-a-chip device can be realized by trapping ECs in a wall. The wall-trapping method is adequate for constructing endothelial barrier-based models. This method is based on perfusing cells through microfluidic channels that contain a porous membrane, an extracellular matrix (ECM), or a hydrogel. The seeded cells are then fixed in the sidewall of the channels to form an endothelial barrier.

If a porous membrane is used, it is constructed using the microfabrication techniques described previously, and generally, PDMS is regarded as a suitable choice for the membrane material. Indeed, the membrane can culture more than one type of cell. Therefore, it can be used to study cell–cell interactions, although full contact between both sides of the cell cannot be assured because they are partially covered with the membrane. Similarly, van Engeland et al. [56] considered the membrane as an internal elastic lamina and co-cultured ECs on the upper side of the microfluidic and vascular smooth muscle cells (VSMCs) on the other side. The elastic properties of PDMS allowed the examination of the EC-VSMC interaction and signaling under hemodynamic conditions, as well as in different mechanical stretching and relaxation states of the membrane. Questioning the optimality of the dimensions and number

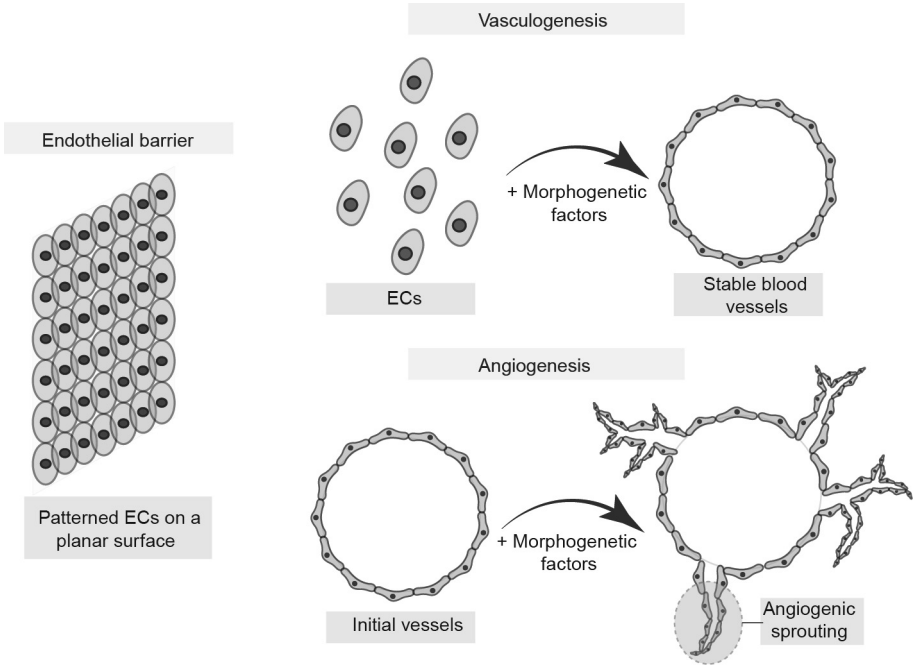

Fig. 4. Schematic of the vasculature models, including the endothelial barrier model, the vasculogenesis model, and the angiogenesis model [40,41].

of pores in the membrane is essential for designing porous membranes. Pore size has a direct influence on permeability and nanoparticle transmigration. Thus, it affects drug migration and other biological phenomena, such as tumor metastasis [57]. Overall, however, the wall-trapping method based on a porous membrane faces a marked limitation because the membranes are generally planar and lack the hollowed aspect of *in vivo* vasculature.

Another option besides a porous membrane is a hydrogel to trap seeded cells. Generally, a collagen or fibrin gel is chosen to create an endothelial wall, which can be constructed using an ECM gel. The use of hydrogels is advantageous for creating lumenized channels. Although the fabrication of a hollowed structure remains challenging without resorting to bioprinting methods, a tubular object such as a needle can be used as a pattern to be removed later in the process after crosslinking the hydrogel [58]. Additionally, the hydrogel-based wall-trapping method allowed full interaction with the surrounding cells without utilizing an intermediate membrane. Although the wall-trapping method is primarily used in the endothelial barrier model, Pauty et al. [59] were able to use it for the angiogenesis model. The research team used a PDMS chip to support the collagen gel, where a bovine serum albumin (BSA)-coated acupuncture needle was inserted and withdrawn to generate a hollowed microchannel structure. Human umbilical vein endothelial cells (HUVECs) were injected and trapped in the microchannel walls. Next, using vascular growth factors, sprouting was induced from the initial vascularized microchannels. This method has been demonstrated to be an efficient tool for studying antiangiogenic drugs, in addition to testing angiogenesis and the permeability of the vascular structure.

Finally, the wall-trapping method can be considered a rapid option to recreate planar or hollow vasculature *in vitro*, using an elastic membrane or a hydrogel to trap the ECs. However, current methods for hollowing the supporting hydrogel are not accurate and cannot overcome precise geometric and dimensional constraints. Moreover, the cell seeding process generates high shear stress, which harms the trapped cells.

2.3.1.2. Microencapsulation method. Using microfluidic chambers or microchannels to encapsulate ECs under morphogenetic conditions is another method used to recreate the vasculature without applying high shear stress on the cells. The chamber encapsulation method is generally referred to as the self-assembling or self-morphogenesis method because the encapsulated cells spontaneously start forming vasculature under precisely well-defined microenvironmental conditions. Therefore, the current method is adequate for producing vasculogenesis and angiogenesis models.

Generally, cell microencapsulation is followed by the injection of a growth factor to promote vascular sprouting and formation. Vascular endothelial growth factors (VEGFs) are broadly used in this method, as well as a few other factors, including fibroblast growth factors (FGFs). FGFs not only exert an effect on blood vessel formation, but also on all other cell types that are important for the formation of arterial vessels [60]. Nevertheless, FGFs are still not well evaluated and are rarely used in vascularized organs-on-chips. Angiopoietins (ANGs) are also added to stabilize (ANG-1) or destabilize (ANG-2) the vascular structure. Campisi et al. [61] cultured human induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) in a microfluidic device. The device was supplemented with VEGF, leading to the successful creation of a vascularized network and an effective platform allowing the triculture of iPSC-ECs, pericytes, and astrocytes to mimic the complex structure and microenvironment of the blood–brain barrier (BBB). The encapsulation of ECs under morphogenetic conditions usually generates ubiquitous vascular networks with unexpected sprouting patterns, which is a substantial disadvantage for constructing an organ-on-a-chip intended to imitate precise and accurate tissue structure and function. Several attempts to achieve a controllable vasculogenesis direction have been conducted and realized by applying microfluidic forces. More precisely, three main forces are involved in shaping the newly formed vasculature by controlling the radius, length, and thickness of microvessels. These forces include shear stress, which is parallel to the tissue surface and is induced by flow characteristics, such as the viscosity and velocity of the perfused fluid, circumferential stress tangential to the tissue surface, and axial stress, which is generated by intraluminal pressure [62]. In addition to the biomechanical factors, many other undefined factors can influence the shapes produced by vasculogenesis and angiogenesis; thus, the morphogenetic factors are still not considered efficient tools for recreating an exact structure.

2.3.2. Bioprinting strategy

Deposition of tissues are generally realized by an emerging biofabrication technique called bioprinting, a newly developed additive manufacturing process that adds biomaterials layer by layer in different ways [63]. The main advantage of bioprinting is its cost-effectiveness and versatility; it is also considered a time-saving technology [64,65]. Therefore, using this technology makes it possible to recreate the ubiquitous 3D structure of the entire vascular network. Bioprinting is a polyvalent strategy that allows the efficient construction of the three previously mentioned models. Indeed, five bioprinting methodologies currently exist, and each has its constraints and applications (Fig. 5).

2.3.2.1. Inkjet-assisted bioprinting. Inkjet technology is a drop-on-demand (DOD) process based on actuating a nozzle with heat or piezoelectricity to put droplets on a stage controlled in 3D. Inkjet bioprinting is usually used because it is a cost-effective method and can maintain high cell viability due to the low shear stress applied on cells (a consequence of the low viscosity-materials used, such as fibrin and collagen) [66]. Nevertheless, this method has low precision and structural integrity. Although droplet-based bioprinting methods are generally inadequate for manufacturing a vertical structure, Hewes et al. [67] successfully achieved a free-standing vasculature in a fibrin matrix using a piezoelectric nozzle. However, the inkjet bioprinting method is not suitable for biofabricating the vasculature because of the need for high structural stability and complexity. Thus, very few inkjet-bioprinted vascularized *in vitro* models can be found in the literature.

2.3.2.2. Laser-assisted bioprinting. To achieve high-speed and high-resolution bioprinting, researchers [68–70] have developed another DOD method, a laser-assisted method in which an energy-absorbing layer carries a layer of the printed biomaterial to be irradiated by a laser source to construct the desired structures. However, the laser-assisted method is not broadly used compared to other bioprinting methods owing to its low structural integrity and scalability and the limited options for adequate biomaterials. This method is generally applied to 2D cell printing; thus, DOD methods are commonly excluded when a vascularized tissue is bio-fabricated. Nevertheless, in a fascinating study, Xiong et al. [71] demonstrated the versatility of this method by creating a freeform bifurcated tubular structure that could potentially serve as a structure for *in vitro* vascular networks.

2.3.2.3. Micro-extrusion bioprinting. The micro-extrusion method is based on pushing the biomaterial through a nozzle under compression. This pressure can be either pneumatic or mechanical [70–74]. The micro-extrusion method is characterized by continuous deposition, feasibility, and good compatibility with several biomaterials. Finally, choosing this method comes at the cost of high shear stress applied to the cells. The flexibility of micro-extrusion-

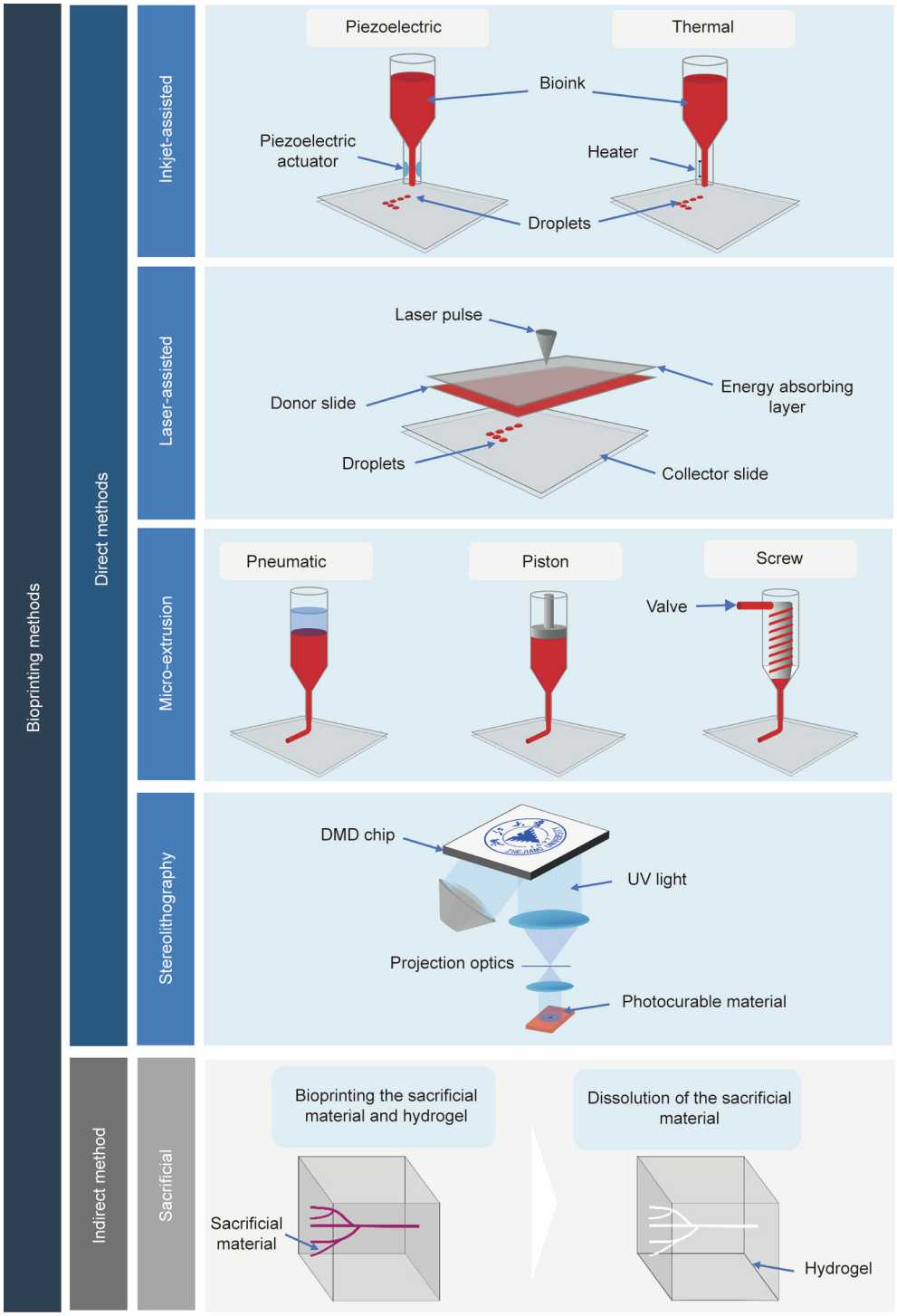

Fig. 5. Schematic of different 3D bioprinting methods. DMD: digital micromirror device.

based bioprinting is evident in its ease of integration into the production process, from printing a single part of the microchip device to a complicated system. Lee and Cho [27] created one of the first one-step fabrication strategies to manufacture a liver with a planar endothelial barrier. The choice of printing material is a crucial initial step; therefore, PDMS was compared to poly(ε-caprolactone) (PCL) on two levels. The team found that the hydrophobicity of both materials was almost equal when measuring their contact angle side by side with a water droplet. The second level was protein adsorption, which generally seems to be neglected, although it is essential in maintaining the medium composition in an "on-chip" device. PCL has comparably low protein absorption, making it more suitable for this type of application, regardless of its low optical transparency. The one-step fabrication method enables spatial heterogeneity and does not require a secondary cell-seeding process as in stereolithography (SLA)-based fabrication methods.

As in standard micro-extrusion bioprinting, embedded bioprinting is an extrusion-based method that uses a supporting material to stabilize the extruded structure and counteract the gravitation

to construct spatially complex architectures. Bhattacharjee et al. [75] used a granular gel as a bioprinting medium, with a tip injected to trace the spatial path of the microvasculature. After injection, the support material was rapidly solidified to trap the embedded material. Many materials, such as silicones, hydrogels, colloids, and living cells, can be injected using this approach; it is also considered one of the most structurally stable biofabrication methods. The classical embedded bioprinting method is a new embedded method that takes advantage of the structural stability of the classical method and creates a freeform structure with a reversible supporting material [76]. This method is called the free-form reversible method or simply the FRESH bioprinting method, and consists of bioprinting tissues in a specifically chosen temporary, thermoreversible, and washable support. After washing the supporting material, a highly complex and stable structure is obtained. The FRESH bioprinting method is thus regarded as an acceptable and advantageous method for constructing a ubiquitous structure of vasculature [77].

2.3.2.4. Stereolithography bioprinting. SLA is a photocuring-based strategy that was first proposed in 1986, which means that it is one of the earliest bioprinting methods [78]. The process begins by selecting a UV-curable material that will later be crosslinking layer by layer [79]. The traditional SLA method provides higher precision and accuracy than other bioprinting methods, but it is also time-consuming because it is based on point-by-point photopolymerization. A recent derivative form of SLA is digital micromirror device (DMD) SLA bioprinting, also known as digital light printing (DLP), which offers higher precision. Indeed, unlike standard SLA, which uses a single mirror, the DMD generally has thousands of adjustable mirrors, allowing better flexibility. Both methods are high-resolution bioprinting modalities and more expensive than other methods. Bioink is another critical choice because of the limited options available in the current literature. Zhang and Larsen [80] adopted poly(ethylene glycol) diacrylate (PEGDA, MW 700) to fabricate a perfusable vascular network. The platform was quite structurally stable, with a perfusion lifetime of at least seven days. Another fascinating and recent study by Grigoryan et al. [81] using DLP proved the complex structure biofabrication potential of this method, for creating 3D intravascular topologies and multivascular networks. The research team achieved a monolithic lung-mimetic perfusion system inspired by the alveolar sac, which is surrounded by a fully functional vascular network, to demonstrate the integrity of the proposed technology. The hydrogel used was a mixture of PEGDA and gelatin-methacryloyl (GelMA) obtained from an optimized passive micromixer. This approach can be regarded as a revolutionary method for constructing vascularized organs-on-chips.

2.3.2.5. Sacrificial bioprinting. Another bioprinting approach, sacrificial bioprinting, can be considered an indirect bioprinting method because of the need for a post-bioprinting technique to remove the fugitive bioink that is was initially directly printed, followed by a different hydrogel matrix that supports the structure [82,83]. The sacrificial bioprinting method is an ideal method for constructing lumenized vascular networks [63]. Ji et al. [84] proposed a novel modified bioprinting approach that combines the application of a photocurable hydrogel with the sacrificial bioprinting method. Photocuring was carried out throughout the process by partially curing a freshly printed layer and executing full curing immediately after achieving the direct printing of the platform that would later be immersed in phosphate-buffered saline (PBS) to dissolve the sacrificial material. This approach slightly affects cell viability and enhances and strengthens the mechanical properties to produce a robust platform.

Table 1
Advantages and disadvantages of various bioprinting methods.

Bioprinting techniques	Advantages	Disadvantages	Refs.
Inkjet-assisted bioprinting	Medium resolution, medium accuracy, rapid, low cost	Requires low-viscosity materials, low structural integrity, inferior mechanical properties, nozzle clogging	[64,65]
Laser-assisted bioprinting	Medium resolution, wide range of printable materials, high accuracy, high cell density, nozzle free	Low structural integrity, inferior mechanical properties, heat harms cells, time-consuming, high cost	[66–79]
Micro-extrusion bioprinting	Superior mechanical properties, wide range of printable materials, high cell density, rapid, medium cost	High shear stress, low resolution, low accuracy, nozzle clogging	[70–75]
Stereolithography bioprinting	High resolution, high accuracy, no shear stress, nozzle free	Limited range of materials, ultraviolet radiation harms cells, time-consuming, high cost	[76–79]
Sacrificial bioprinting	High structural integrity, superior mechanical properties, adequate for hollowed constructs, medium cost	Long fabrication process, low resolution, low accuracy	[80–82]

These various bioprinting methods have their advantages and disadvantages (Table 1) [64–82] and have been continuously explored and developed. The choice of a suitable method for the targeted organ-on-a-chip model depends on several parameters, including the cell types, tissue structure, and most importantly, the primary functions of the organs. Understanding the fabrication process of a vascularized organ-on-a-chip is insufficient for perceiving its integrity using the targeted organ model. Studies have aimed at the biofabrication of human tissues and organs differently owing to the particularity and distinctiveness of each of the functions and structure of the organs. Therefore, considering the critical role that the vasculature plays in real organs and tumor growth, selecting a suitable biofabrication process for a vascularized organ/tumor-on-a-chip is complicated. The only way to attain a thorough understanding of how to construct an efficient model is to review different methods that have been integrated for each specific organ model. Thus, it is necessary to highlight recent developments that have been achieved in vascularized organs-on-chips.

3. Reproducing the vasculature in organs- and tumors-on-chips

3.1. Organs-on-chips

The recreation of vasculature *in vitro* is critical for constructing *in vivo*-like organs because the vasculature plays a vital role in maintaining the properties and functions of tissues. Vascularized

in vitro organs have been achieved for several organs-on-chips, but most importantly for the lung, liver, skin, heart, BBB, and kidney.

3.1.1. Lung

The lung is the primary source of oxygen in the human body; it exchanges gas between the environment and the inner vascular system based on the continuous dynamic movement of the diaphragm. Deep in the lungs, the pulmonary alveoli are found at the bronchioles, where they perform the critical function of gas exchange. During pulmonary alveolar expansion, a fragile layer of alveolar epithelial cells facing the inhaled air allows dioxygen passage to the ECs and then to the capillaries. During exhalation, carbon dioxide is expelled.

The earliest on-chip model was a lung-on-a-chip model developed by Huh et al. [85]. The device could co-culture different cells and mimic the mechanical expansion and contraction of the alveoli (Fig. 6(a)) [85]. The device comprised a porous membrane coated on both sides; by ECs on the upper side and by alveolar epithelial cells on the lower side. The vascularization model is an endothelial barrier model that uses the cell seeding method on a porous membrane. Simultaneously, the chip was used to simulate drug toxicity-induced pulmonary edema with breathing-like motion. In another study, vascularization was demonstrated to be achievable in a lung-on-a-chip using cell seeding on an ECM wall (Fig. 6(b)) [86]. Zhang et al. [86] fabricated a microfluidic device containing three microchannels that supported the alveolar epithelial cells, ECM, and HUVECs. On the side of the lung containing the epithelial cells, TiO_2 and ZnO nanoparticles were perfused to test their toxic effects on the vascularized lung model. The ECM-based cell seeding method provided better cell–cell and cell–matrix interactions than the membrane-based cell seeding method; however, the membrane-based method showed great potential for recreating the mechanical dynamics of an actual lung.

3.1.2. Liver

The liver, considered an organ and the largest gland in the human body, executes several principal functions that regulate the proportion of different indispensable secreted chemicals and components such as cholesterol, triglycerides, and bile [87]. The liver is organized into an almost identical hexagonal lobe, called a lobule, supplied by blood flow from the hepatic artery and portal vein to the central vein, and bile flow goes through the lobule to the bile duct. More specifically, the lobule can be simplified to a representative unit known as the hepatic acinus. The liver comprises two different cell types: hepatocytes and nonparenchymal cells, including hepatic stellate cells, hepatic sinusoidal ECs, and Kupffer cells.

The construction of *in vitro* liver models serves as a fascinating application field for bioprinting methods, which allow precise deposition of an undamaged vascularized liver tissue. For instance, a vascularized liver tissue can be achieved using an extrusion-based bioprinting method and opting for an endothelial barrier model. Lee et al. [88] constructed a platform containing an immortalized hepatic cell line (HepaRG) in a decellularized ECM covered by a thin layer of HUVECs (Fig. 6(c)). The device also has an upper channel facing the endothelium barrier (fulfilling the vascularization role) and a lower channel simulating bile flow, making the on-chip device even more accurate. Similarly, in the previously mentioned one-step bioprinted device, Lee and Cho [27] used a bioprinted PCL chip to co-culture hepatocytes in a collagen hydrogel with ECs forming an endothelial barrier (Fig. 6(d)). The vascularized one-step bioprinted liver-on-a-chip was also compared to 2D *in vitro* models in terms of urea synthesis and albumin secretion to prove the validity and efficiency of the device. In several studies, vascularized livers-on-chips were also realized using microfluidic methods, yet the cells were always exposed to shear stress, which affected their performance.

3.1.3. Skin

The largest organ, the skin, plays a vital role in protecting the body from harmful external factors. Human skin is composed of three layers, starting with the epidermis, which is the outer layer of the skin and is primarily composed of keratinocytes and melanocytes. The second layer is the dermis, which is composed of fibroblasts, macrophages, and mast cells. Finally, the hypodermis is dedicated to fat storage and contains a high percentage of fibroblasts and macrophages. Each layer has specific functions, and all of them permanently interact with the vasculature [89]. Thus, the development of an efficient *in vitro* skin model is strongly dependent on the development of adequate *in vitro* vasculature.

Achievement of a reliable vascularized skin-on-a-chip device can be realized by either the angiogenic or endothelial barrier model. Jusoh et al. [90] demonstrated the constructability of the angiogenesis-based vascular skin model by integrating keratinocytes and HUVECs under released pro-inflammatory factors, which later caused angiogenesis of the blood vessels (Fig. 6(e)). Consequently, the microfluidic platform was shown to be useful for testing the effect of chemical irritants such as sodium lauryl sulfate and steartrimonium chloride on the skin. Further research work, led by Mori et al. [91], focused on the importance of the perfusion ability of the vascular channel in an *in vitro* skin model; the fabrication of a perfusable vascularized model was regarded as a substantial limitation, although necessary. The vasculature was then fabricated by adopting an endothelial barrier model and using a hydrogel-based cell seeding method. The skin-equivalent platform consists of a thin layer of keratinocytes, fibroblast-laden collagen, and a cylindrical endothelial barrier that plays the role of the vascular channel.

Additionally, in a fascinating study, HaCaT cells were co-cultured with ECs and fibroblasts, which were tested with tumor necrosis factor-alpha and caused skin inflammation and edema (Fig. 6(f)) [19]. The three cell layers were cultured based on the cell barrier model and the porous-membrane-based cell seeding method. Overall, vasculature integration provides more efficient drug testing platforms, although bioprinting methods can still be regarded as a potential tool for the co-culture and precise deposition of several cells.

3.1.4. Heart

The heart is a pump that sends blood carrying nutrients, oxygen, and metabolic waste to the rest of the body. It is a continuously active muscular organ with a high need for energy supply and a compact structure. The heart wall comprises several layers, including the endocardium, which is the inner layer in direct contact with the pumped blood inside the heart chambers; the superficial layer is called the pericardium, and most importantly, the myocardium, which is the largest and most rigid layer. The myocardium plays an essential role in generating the aerobic pumping movement; therefore, the oxygen supply must meet the energy requirement in adequate amounts with a well-distributed blood supply. The myocardium is generally targeted when reconstructing the heart because *in vitro* it has a direct role in the contraction/relaxation phases.

In an early study, Chen et al. [92] constructed a microfluidic platform supporting valvular ECs and valvular interstitial cells embedded in GelMA. The device was separated into two channels by a porous membrane, which collected ECs and formed an endothelial barrier (Fig. 6(g)) [92]. More specifically, vasculogenesis of HUVECs occurred in bioprinted microfibers before the tissue was seeded with neonatal rat cardiomyocytes to form an endothelialized myocardium tissue. A perfusion bioreactor was

later added to achieve a vascularized myocardium-on-a-chip model.

3.1.5. Blood–brain barrier

The BBB is a highly accurate semipermeable brain component connecting the central nervous system to the peripheral nervous system. It is composed of a tight layer of ECs surrounded by astrocytes and neurons. The ECs are attached to intercellular connections (proteins) and divide the connections into tight junctions, adherens junctions, and desmosome regions. The passage of substances from the blood is restricted by the ECs, which are more selective than in any other capillary in the human body. The endothelial layer is covered by another layer of mural cells, VSMCs, and pericytes. The capillaries are surrounded by two types of extracellular matrices: the vascular basement membrane, which is secreted by ECs and pericytes, and the parenchymal basement membrane secreted by astrocytes. In addition to neurons, astrocytes surround both layers with their astrocytic feet, which provide biochemical support to these cells.

Although the Transwell model is regarded as an efficient method for mimicking the BBB, it is still a static platform that cannot allow a 3D dynamic culture of cells. Ahn et al. [93] adopted the classic structure of the vascularized organ-on-a-chip, a porous membrane-based structure, to create a microfluidic BBB-on-a-chip platform (Fig. 6(h)). The platform was supported in the upper channel by human brain microvascular endothelial cells (HBMECs) and by human astrocytes (HAs) in the lower channel. The high precision of the model was manifested in its ability to distribute 3D screen nanoparticles. However, the complex structure of the brain, specifically the BBB, does not allow it to be simplified to the co-culture of astrocytes and ECs. Therefore, the device can only be complementary to an *in vivo* model. In a previous study, Brown et al. [94] attempted a successful recreation of the BBB with an almost comprehensive microenvironment (Fig. 6(i)). The platform contained astrocytes, neurons, and pericytes supported in the 3D ECM together in one compartment and ECs in another compartment. This novel platform offers a potential cell–cell interaction environment due to the multicultural compartments that help to ensure accurate human BBB modeling.

3.1.6. Kidney

Kidneys are vital organs that purify blood received from arteries to remove waste and regulate nitrogen, electrolytes, water, and other organic solutes. A single kidney contains approximately one million nephrons, including glomeruli and tubules. Both the glomeruli and the tubules have specific functions. Studies mimicking the kidney have either been achieved as glomeruli-on-a-chip or tubules-on-a-chip [28,95].

The achievement of an *in vitro* kidney is based on the recreation of the renal endothelial–epithelial exchange interface. This realization interface is a tremendous challenge that requires a selective choice of materials and dimensions. A recent on-chip device, the human renal vascular–tubular unit (hRTVU) developed by Rayner et al. [96], integrated a thin collagen membrane to create an exchange interface instead of the traditional PDMS or polycarbonate membrane, because of the limitations of the latter in supporting the incorporation of cells into the bulk matrix. The device recapitulated the *in vivo* dynamic conditions of the kidney using a cell-remodelable hydrogel and customizable perfusion flow. As a result, the hRTVU showed valid quantification of the selective reabsorption of albumin. The device displayed a decaying flow profile in addition to considerable construction issues owing to the lack of precision in the assembly. Clearly, device simplicity is a critical parameter that must be considered. For instance, Homan et al. [97] developed a simplified 3D-printed device housing using PDMS. The research team opted for organoid aggregates to recreate the functional complexity (Fig. 6(j) [97]). Perfusable vasculature was created using the vasculogenesis model. The effect of perfusion flow on the vascularization and maturation of kidney organoids was unclear before this study and needed more attention. The team found that the flow enhanced the vascularization and maturation of the organoid in the tubular and glomerular compartments. However, the formed vasculature cannot ensure flow perfusion through the microvascular networks and needs to be addressed to improve the device.

3.2. Tumors-on-chips

Throughout history, cancers have been fatal, and it is one of the few diseases that humans are yet to find a cure for. The data on cancer is shocking [98]. This complicated disease caused 9.56 million deaths worldwide in 2017, making it the second cause of death after cardiovascular diseases (17.79 million deaths) [99]. Cancer is characterized by unregulated growth and invasion of cells from their original sites. Conventionally, it is considered a sequence of genome mutations, resulting from several factors that we still do not entirely understand.

The deadliest stage of a tumor occurs when it spreads in the human body. To metastasize, the tumor needs a link with other organs; that is, the role of a pre-metastatic niche, which helps to seed cancer cells and encourages metastatic outgrowth and finally a tumor niche [100]. A tumor niche is a complicated heterogeneous tumor microenvironment (TME) that provides favorable growth conditions for cancer cells [101–106]. It includes several cell types, such as stromal cells, fibroblasts, and ECs. For this reason, mimicking tumors is a challenge, which has forced scientists to innovate and simplify the tumor niche to a more analyzable model. Presently, four tumor models exist: counting the 2D monolayer model, the 3D static model, the tumor-on-a-chip model, and the animal model. Various studies have shown that the tumor-on-a-chip model has several advantages over other models, such as recreating a 3D structure under dynamic conditions with easy access and control. Thus, it has attracted more attention and attempts have been made to create new, more accurate tumor-on-a-chip platforms [5,107–109].

Blood vessels are necessary to provoke angiogenesis and supply the metabolic demand of malignant cells, and this emphasizes the importance and actual need of integrating vasculature into tumors-on-a-chip to achieve a vascularized tumor-on-a-chip model. In addition, vascularized tumors-on-chips have been realized using only endothelial cell networks, without the capacity for perfusion [5]. Mass transport is required principally for living cells to deliver nutrients and oxygen and remove metabolic byproducts *in vivo*. Furthermore, 3D cells aggregate, permitting cell–cell and cell–matrix interactions, to form either spheroids or organoids.

3.2.1. Vascularized tumor spheroids on-chip

For over 40 years, spheroids have been used as tumor models because of their similar narrow shape and cellular density in real *in vivo* tumors. Arguably, fabricating spheroids is the most feasible way to achieve a 3D cellular structure [110–118]. Additionally, it is undeniable that integrating perfusion into microfluidic platforms is advantageous. Additionally, it is undeniable that integrating perfusion into microfluidic platforms is advantageous. A fusion between such microfluidic platforms and the spheroid culture method can lead to a more efficient platform.

This fusion approach was applied by Sobrino et al. [119] in a simplistic *in vitro* vascularized micro-tumor (VMT) PDMS platform without pre-patterned microvessels and without requiring any pump or tubing (Fig. 7(a)). The platform permits delivery of nutrients through microvessels, supporting breast and colorectal cancer.

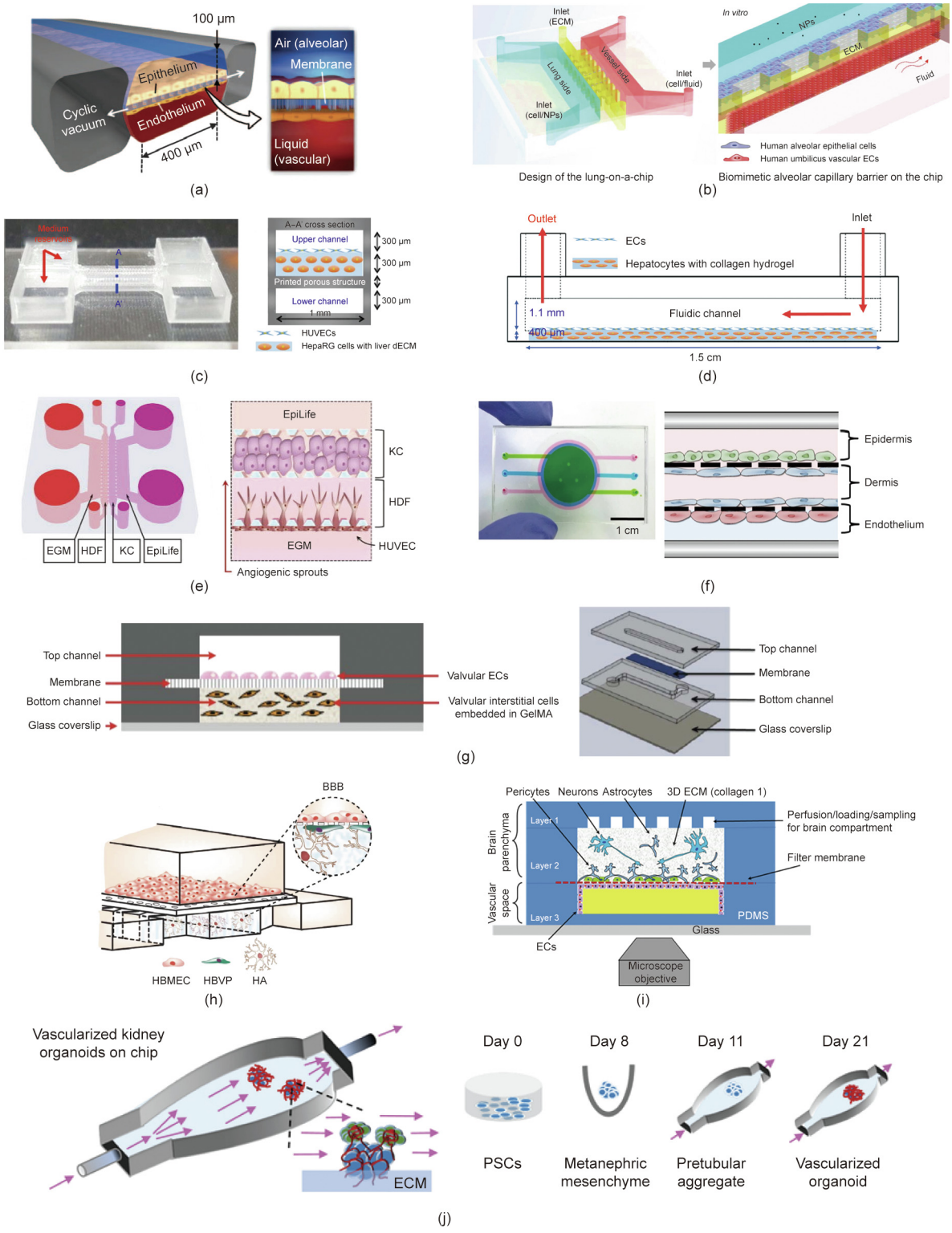

Fig. 6. Vascularized organs-on-chips. (a) Schematic of the dynamic lung-on-a-chip. (b) Schematic of a lung-on-a-chip utilizing an ECM wall. NPs: nanoparticles. (c) A liver-on-a-chip model supporting the liver microenvironment and biliary system. dECM: decellularized ECM. (d) Schematic of a one-step fabricated liver-on-a-chip. (e) Schematic of angiogenesis-based vascularization in a skin-on-a-chip, which included endothelial growth medium (EGM), human dermal fibroblasts (HDFs), keratinocytes (KCs), and an EpiLife medium [90]. (f) Vascularized skin-on-a-chip containing HaCaT cells, ECs, and fibroblasts [19]. (g) Schematic of a heart-on-a-chip supporting valvular endothelial and interstitial cells separated by a porous membrane. (h) Schematic of the co-culturing BBB model with human brain vascular pericytes (HBVPs), HBMECs, and HAs [93]. (i) Layout of a neurovascular unit [94]. (j) Schematic of renal organoid on-chip with a representation of the formation of the vascularized organoid. PSCs: pluripotent stem cells [97]. (a) Reproduced from Ref. [85] with permission of the American Association for the Advancement of Science, ©2012; (b) reproduced from Ref. [86] with permission of the Royal Society of Chemistry, ©2018; (c) reproduced from Ref. [88] with permission of IOP Publishing Limited, ©2019; (d) reproduced from Ref. [27] with permission of the Royal Society of Chemistry, ©2016; (g) reproduced from Ref. [92] with permission of the Royal Society of Chemistry, ©2013.

Therefore, a test was carried out to analyze the response of colorectal cancer VMTs to standard anticancer therapies by screening Food and Drug Administration (FDA)-approved drug combinations. The triple-negative breast cancer line (MDA-MB-231) was

examined for standard-of-care chemotherapies and the VMT platform showed high sensitivity to antiangiogenic and vascular disrupting agents. Therefore, the platform represents an efficient tool to identify reagents that target tumor cells directly or indirectly through their vasculature effects.

Organ-on-a-chip accuracy is generally related to the number of cell types and interacting organs that reflect the real tumor niche. Thus, Chung et al. [120] considered microfluidic devices simulating only stroma–cancer interaction as too simplified to provide systemic insight into the complex structure (Fig. 7(b)). Therefore, the research team proposed a novel approach which involves mimicking both angiogenesis and lymphangiogenesis in the TME. Similarly, Mannino et al. [121] developed an *in vitro* platform to recapitulate the interactions that occur *in vivo* between cancer cells, ECs, and immune cells (Fig. 7(c)). They targeted diffuse large B-cell lymphoma and engineered an easily accessible and economical hydrogel-based lymphoma-on-a-chip. This particular fabrication method favored the ability to extract the cellular components of the lymphoma-on-a-chip model for post-treatment analysis. Additionally, this platform enables easy visualization of spatial interactions between different components.

Another solution for better visualization was proposed by Paek et al. [122], who completed a cell culture on an open-top microdevice to facilitate the integration of other specialized tissues with the vascular network. Moreover, the engineered microdevice contained a vascularized adenocarcinoma constructed by incorporating tumor spheroids into an *in vitro* model. The fabrication process of the device combined microfluidic 3D cell culture with vasculogenesis to form perfusable blood vessels *de novo*.

The interaction between healthy and tumorigenic tissues is another critical parameter for establishing a realistic environment. Ozkan et al. [123] developed a model supporting both tissue types with a vasculature formed by a cylindrical endothelial wall covered separately by breast tumor cells in the first zone and liver cells in the second zone (Fig. 7 (d)). The two zones were connected in series to test drug and nanoparticle transport and development in each zone. The device successfully simulated the interaction between several healthy and tumorigenic tissues.

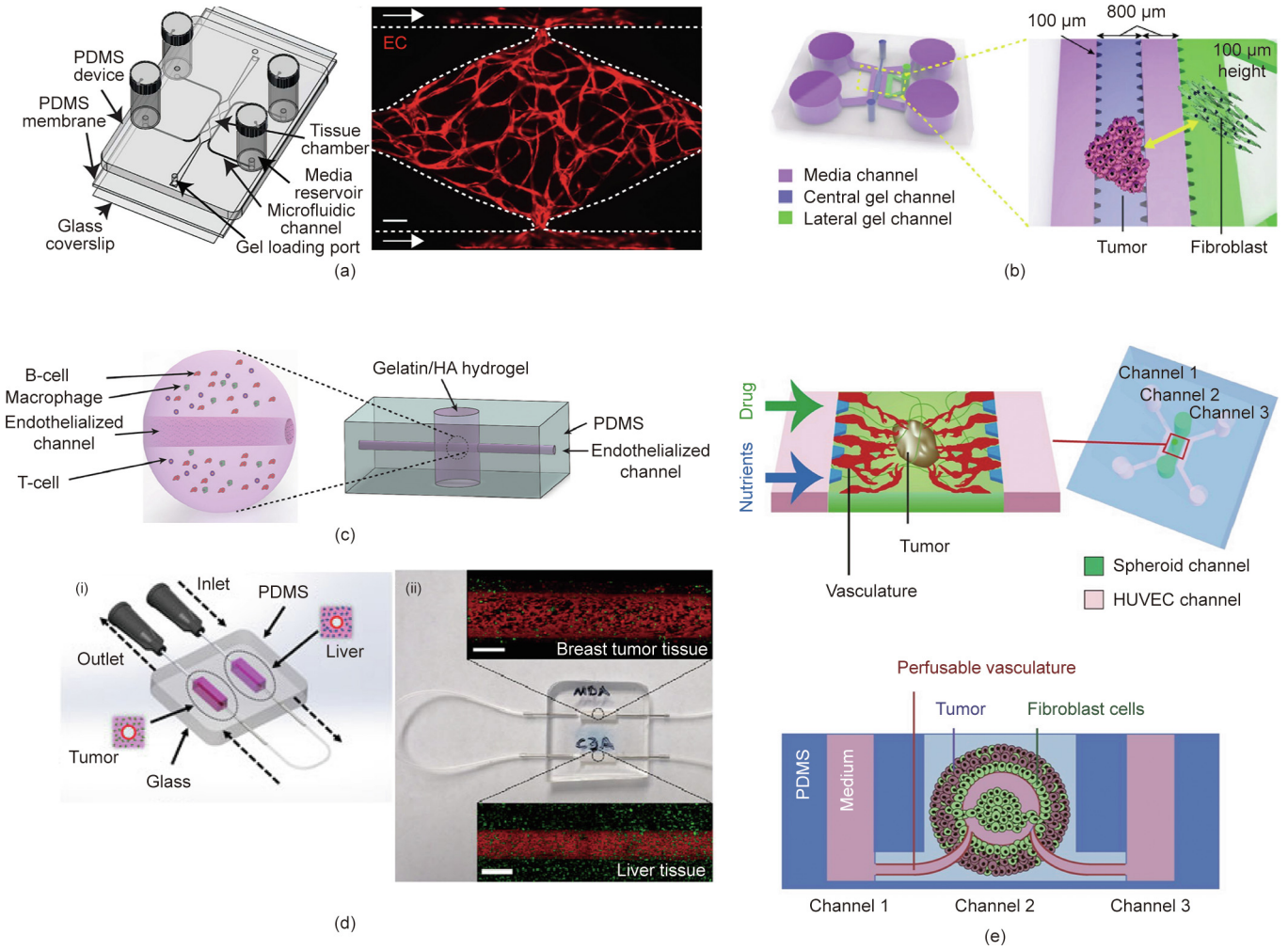

Fig. 7. Vascularized tumor spheroid on-a-chip. (a) Schematic of the vascularized tumor-on-a-chip device with a confocal image of a representative tissue chamber containing a fully developed vascular network during its seventh day [119], scale bar: 100 μm; (b) schematic of a microfluidic device allowing an examination of tumor–stroma interactions; (c) schematic of a diffused large B-cell lymphoma hydrogel in a PDMS device; (d) schematic of a platform with healthy liver tissue and TME (i) and a confocal image of the preconditioned tumorigenic and healthy vessels (ii), the scale bar is 500 μm; (e) schematic of a microfluidic platform containing two human umbilical vein endothelial cell channels, channel 1 and channel 3, one tumor spheroid channel, channel 3, and a cross-section of the device showing the configuration of the tumor, fibroblast cells, and vasculature. (b) Reproduced from Ref. [120] with permission of Wiley-VCH Verlag GmbH and Company KGaA, ©2017; (c) reproduced from Ref. [121] with permission of the Royal Society of Chemistry, ©2017; (d) reproduced from Ref. [123] with permission of John Wiley and Sons, ©2019; (e) reproduced from Ref. [124] with permission of Elsevier, ©2020.

Until now, almost all the proposed approaches have failed to achieve an evaluation of drug efficacy at times surpassing 24 h, which is necessary to simulate the mechanical stress applied to the tumors. Nashimoto et al. [124] recapitulated the long-term perfusion of a pressure-driven flow through a vascular network in an *in vitro* device (Fig. 7(e)). The long-term perfusion culture reinforced the proliferation of tumor cells and suppressed cell death. Similarly, Nie et al. [34] developed a multiscale preconstructed microvasculature fabrication strategy based on the peeling and bonding process, with a double crosslinking strategy. Tumor spheroids were integrated to simulate the interaction between the vasculature and spheroids to validate the approach and demonstrate its flexibility. Finally, the spheroids on-a-chip model offered a wide variety of solutions with impressive studies that showed its efficiency, but the model also can be regarded as a simplistic model that needs to simulate more complex structures and functions of the actual organs.

3.2.2. Vascularized tumor organoids on-a-chip

In 1907, Wilson showed the ability to differentiate siliceous sponges and grow them into identical sponges, which is considered the first and early step of the revolution of the organoids [125]. An organoid is generally defined as a "collection of organ-specific cell types that develops from stem cells or organ progenitors, and self-organizes through cell sorting and spatially restricted lineage commitment in a manner similar to *in vivo*." Organs-on-chips and organoids have been broadly investigated over the last decade. Even though the organ-on-a-chip platform has been considered an over-simplified model, both organs-on-chips and *in vitro* culture promise to push further scientific advances in clinical research.

Interestingly, a slight intersection between the organoids and organs-on-chip models occurs when one or more organoids are merged in a microfluidic platform (Fig. 8) [126,127]. The synergy between these approaches has been illustrated by Lancaster et al. [128] in a platform consisting of human pluripotent stem cells

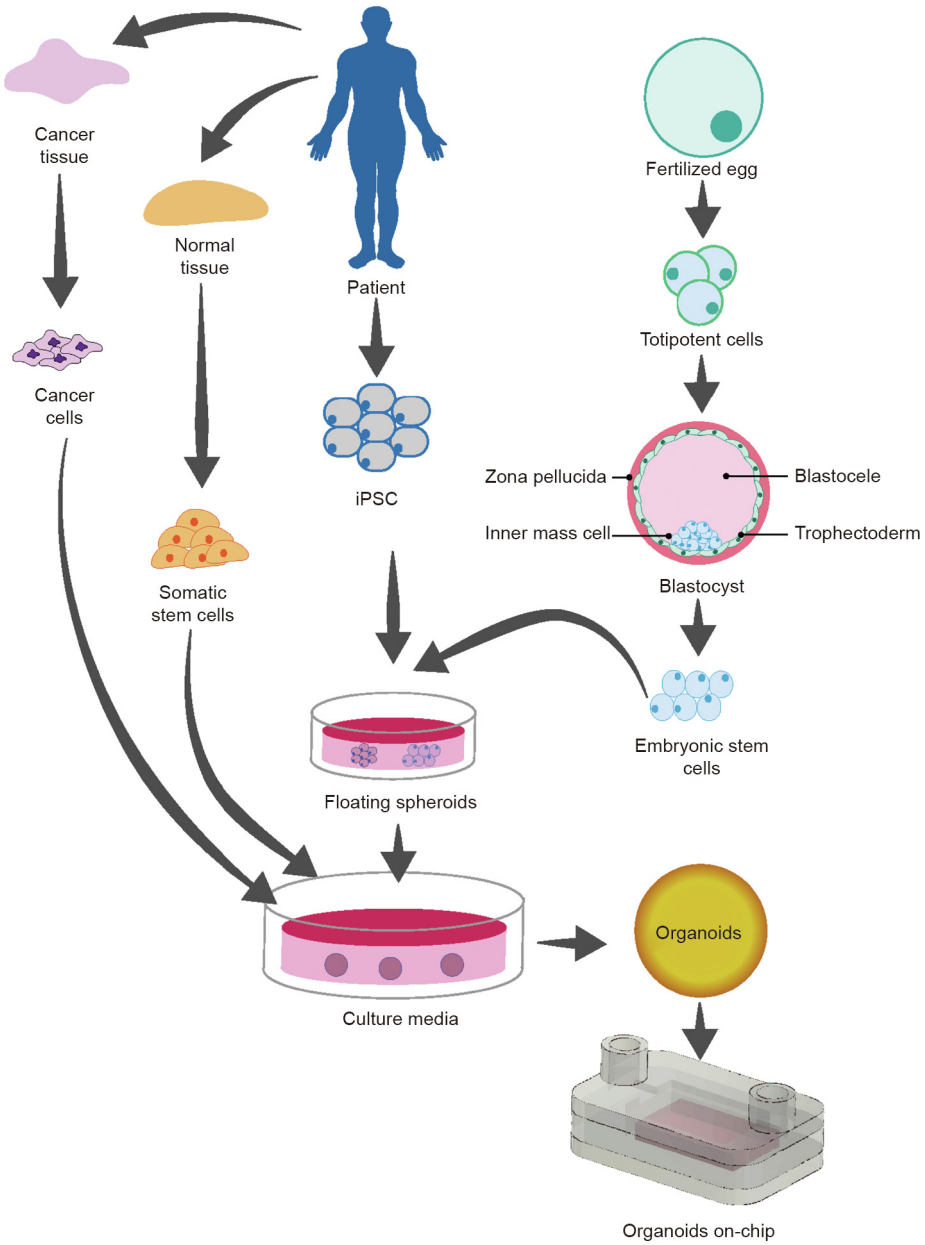

Fig. 8. Schematic of organoids-on-a-chip construction from cancer cells and stem cells [126,127]. iPSC: induced pluripotent stem cell.

attached to poly(lactic-*co*-glycolic acid) (PLGA)-based microfilaments to form microfilament-engineered cerebral organoids. The combination demonstrated better reproducibility and cortical plate formation than either method alone.

Metastasis, a late stage of a tumor, occurs when tumor cells spread through blood vessels from their original place to another location in the body; thus, the recreation of a vascularized model is indispensable to recreating metastasis. Combining tumor organoids on chips with the *in vitro* vascularization approach can result in an accurate tumor-representative model. This challenging dynamic phenomenon was mimicked by Skardal et al. [129] to engineer a metastasis-on-a-chip device. The platform comprises connected PDMS chambers that support colorectal cancer organoids and liver, lung, and endothelial constructs. The device offers a promising opportunity to better understand the metastasis mechanism and help identify intervention targets.

In another study, Shirure et al. [51] constructed a primary breast tumor organoid on-chip model based on angiogenesis-fabricated microvasculature. The microfluidic platform included a structurally stable quiescent vasculature with the ability to support both tumor cell lines and patient-derived tumor organoids in adjacent tissue chambers. The device allows the simultaneous examination of the impact of antiangiogenic agents and chemotherapeutics, which could substantially impact modern clinical approaches.

4. Challenges and future perspectives

The current advances in vascularized organs-on-a-chip represent a promising, fertile field for developing new drug testing platforms. Biofabrication methods remain critical to obtaining the desired physicochemical properties. Nevertheless, current biofabrication technologies still lack spatial controllability and cell printing resolution and accuracy, which are crucial for constructing organotypic vascularized tissues. Several vascular-related diseases are based on microscopic dysfunctions and require precise microscale recreation to obtain a valid and efficient *in vitro* model. Therefore, in addition to the limited accuracy of bioprinting methods, precision can also be regarded as a substantial challenge in constructing vascularized tissue.

Additionally, only a few studies have been able to recreate the ubiquitous 3D vascular network structure using a bioprinting technique. These limitations are primarily related to the insufficient mechanical properties of the bioinks, which generally fail to maintain the desired shape owing to swelling or related rheological properties [130,131]. Structural heterogeneity of the tissue is another fundamental requirement for imitating an *in vivo* vascularized microenvironment, and is an obstacle to the development of *in vitro* models. The voxel-based bioprinting approach is efficient in constructing heterogeneous topologies and could potentially overcome the heterogeneity barrier in the organ-on-a-chip technology [132,133]. Thus, developing new innovative biofabrication techniques and adapting standard techniques to the process and bioink is essential and primordial.

Evaluation of the overall fabrication process is another crucial criterion to consider. Implementing bioprinting in vascularized organ-on-a-chip fabrication allows rapid construction and minimizes the process to only one step. However, currently, one-step fabrication methods cannot determine the structural complexity of the vasculature. Other methods generally require a bonding step, which decreases the precision and limits the creation of a ubiquitous structure in only 2D. The exigency of crossing from a 2D to a 3D structure opens the door to integrating smart materials, specifically four-dimensional (4D)-bioprinted materials [134,135].

Integrating human-induced pluripotent stem cells in tumor-on-a-chip platforms revealed the importance of selecting this cell source, which provides a compatible phenotype for tumor disease modeling with high proliferation capacity. Additionally, recent developments in organoids have improved the accuracy of organ-on-a-chips devices. Therefore, the parallel and synchronized evolution of the organoid models and vascularized organs-on-a-chip could give rise to a more accurate fusion of the two models and enable faster development of personalized medicine.

Finally, the human body comprises several organs that usually interact, and it is difficult to predict these interactions using standard *in vitro* culture methods. The body-on-a-chip enables the control and real-time observation of the interactions between different organs [136]. Merging the perfusable vasculature and other organ tissues in an organ-on-a-chip device improves its integrity into a circulatory system supporting several tissues, that is, a body-on-a-chip. In addition to the perfusable structure of the body-on-a-chip, it requires the creation of a blood-mimetic perfusable medium, which is also considered a substantial challenge. Thus, a universal medium that provides nutrients to different cells needs to be developed to support this technology.

5. Conclusions

Drug testing and disease analysis are important and critical global needs. Fabrication of accurate devices with the functional organization of living cells mimicking real human organs can serve as an effective solution to overcome these needs. In the current review, a synergic approach to the fabrication of the vasculature and organs-on-a-chip has been briefly introduced, confirming the intensive research and development in this field.

Organs-on-chips can generally be manufactured in five consecutive steps. The device can be achieved most successfully using soft-lithography or photolithography. In the organ-on-a-chip field, tissues are often constructed using bioprinting techniques. The vasculature can be integrated into an organ-on-a-chip by applying at least one of the three models: the endothelial barrier model, vasculogenesis model, or angiogenesis model. The application of this synergistic approach has been demonstrated in several domains, but most specifically in tumor culture, which proved that vascularized tumors-on-a-chip have comparatively promising potential for further development.

Ultimately, many studies on the vasculature and organs-on-a-chip have shown not only their importance, but also the colossal number of constraints; these should motivate scientists from different domains to develop new biofabrication methods and achieve a better organ-on-a-chip device with higher accuracy and efficiency.

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Compliance with ethics guidelines

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