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REVIEW ARTICLE

3D bioprinting strategy for engineering vascularized tissue models

Suhun Chae[1], Dong-Heon Ha[1]*, Hyungseok Lee[2,3]*

[1]EDmicBio Inc., Seoul 02458, Republic of Korea

[2]Department of Mechanical and Biomedical Engineering, Kangwon National University, Chuncheon 24341, Republic of Korea

[3]Department of Smart Health Science and Technology, Kangwon National University, Chuncheon 24341, Republic of Korea

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Abstract

Leveraging three-dimensional (3D) bioprinting in the fields of tissue engineering and regenerative medicine has rapidly accelerated progress toward the development of living tissue constructs and biomedical devices. Ongoing vigorous research has pursued the development of 3D *in vitro* tissue models to replicate the key aspects of human physiology by incorporating relevant cell populations and adequate environmental cues. Given their advantages of being able to intimately mimic the heterogeneity and complexity of their native counterparts, 3D *in vitro* models hold promise as alternatives to conventional cell cultures or animal models for translational application to model human physiology/pathology and drug screening. Research has highlighted the importance of *in vitro* models, and a sophisticated biomanufacturing strategy is vitally required. In particular, vascularization is critical for the prolonged survival and functional maturation of the engineered tissues, which has remained one of the major challenges in the establishment of physiologically relevant 3D *in vitro* models. To this end, 3D bioprinting can efficiently generate solid and reproducible vascularized tissue models with high architectural and compositional similarity to the native tissues, leading to improve the structural maturation and tissue-specific functionality. Multiple bioprinting strategies have been developed to vascularize *in vitro* tissues by spatially controlled patterning of vascular precursors or generating readily perfusable vascular structures. This review presents an overview of the advanced 3D bioprinting strategies for vascularized tissue model development. We present the key elements for rebuilding functional vasculature in 3D-bioprinted tissue models and discuss the recent achievements in the engineering of 3D vascularized *in vitro* models using 3D bioprinting. Finally, we delineate the current challenges and future outlooks of 3D bioprinting-based vascularized tissue models.

Keywords: 3D bioprinting; Vascular tissue models; Organ-on-a-chip; Biofabrication; *In vitro* models

***Corresponding authors:**
Dong-Heon Ha
(matt@edmicbio.com)

Hyungseok Lee
(ahl@kangwon.ac.kr)

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1. Introduction

Three-dimensional (3D) *in vitro* models, such as organ-on-a-chip or micro-physiological systems, refer to biomimetic *ex vivo* miniaturized platforms mimicking

the key aspects of human physiology and disease[1,2]. Although many biomedical studies have typically used two-dimensional (2D)/3D cell culture methods or animal experiments, such conventional pre-clinical models face significant drawbacks, including the lack of biological complexity, cross-species discrepancies, and ethical issues[2,3]. Alternatively, 3D *in vitro* models facilitate biomedical experimentations through the inclusion of relevant cell sources and organized biological structures in controlled microenvironments. Therefore, these models have received increasing interests as the next-generation research platform to investigate human (patho) physiology and impact drug discovery pipelines[1,4]. Given their inherent advantage of replicating physiological organ functions in a robust and reproducible manner, humanized *in vitro* models offer intriguing opportunities to replace traditional cell cultures and animal models for clinical translation.

The ultimate goal of 3D *in vitro* models is to reproduce physiologically and biologically realistic human model systems outside the body. To date, substantial progress has been made in the development of *in vitro* models to mimic the natural microenvironment and to understand organ-specific function and pathological behavior[5-7]. However, the current models cannot reliably recapitulate the key structural and physiological properties of their native counterparts. In particular, the lack of vascularization in the engineered tissues is one of the paramount issues[8]. In the human body, the vascular network represents hierarchical organization and serves for the efficient exchange of nutrient and oxygen and for the removal of wastes within and between tissues/organs[9]. In addition, the presence of vascularization in engineered tissues not only maintains cell viability and function, but also supports cross-talk between diverse cell and tissue types, effectively mimicking human biological responses[10]. Thus, engineering functional vasculature is a prerequisite for the successful engineering of physiologically relevant *in vitro* models. Over the past few decades, significant efforts have been devoted to developing 3D vascular tissues that replicate the physiological properties of human vasculature within an *in vitro* biological system by introducing several intrinsic/extrinsic elements[9-11]. Numerous approaches for generating vascularized tissues using various biofabrication techniques (e.g., nano-/micro-fabrication, soft lithography, and replica molding for micro-fluidics) have already been explored[3,12,13]. Recently, self-assembling approaches have been attempted to create tubular structures by systematically modulating the co-assembling components[14-16]; nevertheless, vascularized tissues reflecting the complexity and multifariousness of vascular environments, including anatomical structure, biochemical and biophysical dynamics, and relevant physiological functions, have not yet been successfully engineered.

To tackle this problem, 3D bioprinting has emerged as a promising tool for developing an advanced *in vitro* model with functional vasculature. 3D bioprinting allows for the generation of prescribed biomimetic tissue structures through precise depositions of biomaterials, cells, and biomolecules[17,18]. As each organ has its own unique physiological role and contains distinct cell types and structures, a suitable design, materials, and fabrication strategy are needed to emulate tissue/organ specificity and functionality in vascularized tissue models. A high-precision, multi-material 3D bioprinting system has been considered a promising tool to fabricate tissue models that mimic native tissue structures and reproduce complex biological environments in a tissue-specific manner. In addition, to obtain reliable *in vitro* models, advanced strategy is necessary for providing guidance cues within an organ-mimicking 3D environment, which could further enrich the structural similarity and functional maturity of the engineered tissues[19]. Despite the advances in biofabrication techniques for engineering complex vascular networks, unresolved issues remain, including their poorly defined structural organization, unmet size scale, and immaturity[5,20,21]. In this respect, 3D bioprinting with multi-scale and multi-material fabrication process is useful to achieve robust vascularization in printed tissue models. Given the great importance of systematic interaction between vasculature and engineered tissues in the developmental process, numerous bioprinting methods have been extensively explored to vascularize *in vitro* tissues, including by spatial patterning of vascular precursors or generating a readily perfusable vascular structures.

This review highlights the recent advances in 3D bioprinting strategies for vascularized tissue model development. First, we introduce the main bioprinting approaches for the fabrication of vascular structures and describe the key elements to rebuild functional vasculature in engineered tissues using 3D bioprinting. In the context of engineered tissue vascularization, we focus on delineating the 3D bioprinting strategies that not only build readily perfusable vascular channels, but also incorporate vascular networks into bioprinted tissues via intrinsic/extrinsic induction. Next, we discuss the recent achievements in engineering 3D vascularized *in vitro* models using 3D bioprinting. Finally, the current challenges and future perspectives of engineering 3D bioprinting-based vascularized tissue models are delineated.

Table 1. Major 3D bioprinting strategies for developing vascular structures

Bioprinting strategies	Descriptions	Major benefit in vascularized tissue construction
Coordinated patterning	Spatial arrangement of cell-laden or cell-compatible inks at desired locations to produce 3D cellular construct with inter-connected pre-vascular networks	Coordinated (spatially defined) patterning of desired vascular cell sources and pro-angiogenic factors with high design flexibility of manipulating internal structures and porosity throughout the construct
Sacrificial printing	Deposition of a fugitive ink in any desired geometry, followed by casting and removal of sacrificial material, enabling manual cell seeding to create endothelialized channels	Introduction of physical architectures, such as open and inter-connected pores or perfusable micro-channels, within 3D hydrogel-based constructs with high freedom on designing channel geometries and a wide size range
Embedding printing	Extrusion of designated ink materials into the liquid suspension bath to hold the printed filaments and their desired geometry while printing	Beneficial for improving printability of soft bioink and for increasing structural integrity with high design flexibility and resolution
Coaxial printing	Through a core/shell printing configuration, simultaneous extrusion of different materials to create hollow tubular structures in a single process	Direct printing of freestanding tubular structure with high dimensional flexibility (e.g., diameter, wall thickness, and length) in a uniform size

2. 3D bioprinting strategies to build vascular structures

Numerous 3D bioprinting techniques, such as inkjet-based, laser-assisted, and extrusion-based ones, are being used to develop multi-scale vascular structures. The fundamental principles and characteristics of prevailing bioprinting techniques have already been extensively reviewed elsewhere[22-25]. Among these prevailing techniques, extrusion-based bioprinting is widely employed to fabricate complex hierarchical vascular structures. Thus, here we focus on the application of extrusion-based bioprinting methods. In extrusion-based bioprinting, cells are encapsulated in an exogenous biomaterial ink (i.e., hydrogel), which acts as the supporting matrix. According to the programmed G-code, the designated 3D tissue structure can be created. Once the printing process is completed, the cell-laden construct undergoes solidification to retain its desired shape. The extrusion-based method is preferred over other bioprinting methods owing to its ability to utilize a broad library of biomaterials with high-viscosity inks at a higher cell density. In this section, we outline the prominent extrusion-based bioprinting strategies and elaborate on their principles for vascularized tissue fabrication (Table 1).

2.1. Coordinated patterning

Coordinated patterning strategy refers to the subsequent process of extruding at least one cell-containing ink and one biomaterial ink by iteratively switching between different printing heads and spatially pattering them on demand. Based on the primary mechanisms of vasculogenesis and angiogenesis in the formation of vascular networks[9], coordinated (spatially defined) patterning of vascular cell sources and pro-angiogenic factors allows to build vascularized tissue constructs. Further, through a multi-material printing process, spatial patterning of vascular precursors at desired locations using cell-laden or cell-compatible materials as (bio)inks can produce a 3D cellular construct with inter-connected pre-vascular networks. For example, Jang *et al.*[26] developed multi-cellular and multi-layered constructs through the 3D spatial patterning of vessel-forming cell sources and pro-angiogenic growth factors to achieve a pre-vascularized cardiac patch, resulting in improved cell–cell interaction and differentiation as well as vascularized tissue regeneration. Maiullari *et al.*[27] presented a multi-cellular 3D bioprinting approach to fabricate heterogeneous vascularized cardiac tissue by tailoring the spatial organization of the two cell types, which can facilitate enriched vascular networks. Bioprinted constructs with pre-patterning of vascular precursors can possibly use paracrine signals to enhance cell–cell communication and differentiation capacity, thereby improving the vascularization of the engineered tissue.

Employing the coordinated pattering strategy is beneficial for the precise spatial localization of desired cell types and bioactive molecules and provides high design flexibility by allowing the manipulation of internal structures and porosity throughout the construct. However, the integrated processing of multiple materials poses technical drawbacks, such as cross-compatibility between the materials and rapid solidification for stable construction, all of which require continuous efforts in advancing bioprinting techniques and biomaterials.

2.2. Sacrificial bioprinting

As a top-down approach, sacrificial bioprinting deposits a fugitive material in any desired geometry and subsequently casts it onto another hydrogel. "Sacrificial" ink materials

are only temporarily present during the printing process, and the removal of sacrificial networks can create hollow structures, which enables cells to be seeded to produce lumenized vessels inside a 3D hydrogel. Because the solid sacrificial network can aid in maintaining micro-channel networks, this indirect method has been explored for the 3D bioprinting of vascular channels in engineered tissues. For example, Homan *et al.*[28] presented the sacrificial bioprinting approach to fabricate 3D convoluted tubular structures on customized perfusable chips, where the printed Pluronic F127 (PF-127)-based fugitive ink was removed and the proximal tubule (PT) epithelial cells were seeded to yield an open convoluted tubular channel embedded within a gelatin–fibrinogen hydrogel. A similar method has been adopted by Kolesky *et al.*[29] to create 3D heterogeneous vascularized constructs containing perfusable channels interleaved with vascular supporting cells (i.e., fibroblasts) within a photocurable surrounding matrix (i.e., gelatin methacrylate [GelMA]); this method presents a combinatorial approach for fabricating 3D tissue constructs comprising vasculature, multiple types of cells, and extracellular matrix (ECM). Several sacrificial inks including PF-127[28,29], carbohydrate glass[30], and gelatin[31,32] have been used in this approach, all of which have been shown to successfully fabricate complex 3D vascular structures.

Sacrificial printing strategy allows the introduction of physical architectures, such as open and inter-connected pores or perfusable micro-channels, within bulk hydrogel-based constructs. In addition, this approach provides a high degree of freedom for designing channel geometries with a wide size range and is therefore efficient for generating large-scale channel networks. However, its relatively low printing resolution in channel diameter (>100 μm) is a key obstacle to mimicking micro-scale channels with sizes close to capillary vessels (10–20 μm in diameter)[9].

2.3. Embedding bioprinting

In general, direct deposition of bioinks or biomaterial inks without supporting materials makes the printed tissue constructs prone to collapse or deformation. To address this challenge, embedding bioprinting has been proposed to meet the increasing demand for large-scale and high-precision fabrication. In this technique, ink materials are extruded into a liquid suspension bath (i.e., suspension media or granular hydrogels) according to a pre-defined pattern, thus allowing to effectively dispense low-viscosity bioinks into a support reservoir[6,10,20]. The suspension bath serves as a support agent to hold the printed filaments and their designated geometry while printing, which is beneficial for expanding the deposition ability of soft bioink and increasing structural integrity. A recent technological breakthrough—freeform reversible embedding of suspended hydrogels (FRESH)-based printing technique—allows the freeform fabrication of more complex structures. Lee *et al.*[33] developed a coacervation approach to generate thermo-reversible gelatin micro-particles used as support bath. This FRESH technique significantly enhanced resolution (length scales ranging from a few millimeters to centimeters) with the ability for the precise deposition of soft hydrogels into intricate 3D biological constructs. In this study, they also presented a method to 3D-bioprint collagen bioink using FRESH to re-build the components of the human heart at multiple length scales, from capillaries to the full organ. After printing, the gelatin support bath was mildly removed by placing at 37°C to retrieve the printed construct. Recently, this approach has been adapted to fabricate several 3D vascularized tissues such as cardiac tissues[34], blood vessels[31], and muscles[35].

The embedding bioprinting strategy has tremendous potential for reproducing complex branched structures with various diameters in 3D owing to its advantage of high design flexibility and resolution. In addition, it can broaden the range of applicable bioinks to better mimic the structure and function of the printed tissue. However, the complete elimination of the sacrificial support material in a temporally controlled fashion, the limited range of available supporting materials, and the unavoidable biochemical reaction between bioink and supporting bath material may be major drawbacks.

2.4. Coaxial bioprinting

Coaxial extrusion can be accomplished through a core/shell printing configuration, which simultaneously dispenses two or more flow streams in concentric rings. A coaxial nozzle usually possesses an inner core into which a crosslinking agent or sacrificial material is dispensed; this enables the semi-crosslinking of the outer shell hydrogel to create hollow micro-tubular constructs in a single-step procedure. With the careful selection of nozzle dimensions and dispensing flow rates, coaxial bioprinting can pave the way to the direct printing of freestanding tubular structures with varying wall thicknesses and lumen diameters in a uniform size. Owing to its simplified manufacturing process and scalability, coaxial bioprinting has been increasingly investigated for emulating vascular constructs[36-38]. For example, Jia *et al.*[39] reported the direct construction of organized, perfusable vascular structures using a blended bioink comprising GelMA, sodium alginate, and four-arm poly(ethylene glycol)-tetra-acrylate in combination with a multi-layered coaxial extrusion system, which facilitated the accurate deposition of multi-layered 3D perfusable hollow tubes. As a significant leap forward, Gao *et al.*[40] used a triple coaxial nozzle for fabricating three-layered

conduits with tunable geometry and dimensions by controlling the nozzle moving speed and extrusion flow rate. They designed a tri-layered coaxial nozzle containing sacrificial PF-127 in the core region, endothelial cells (ECs)-laden vascular tissue-derived decellularized ECM (VdECM) bioink in the intermediate region, and smooth muscle cells-laden VdECM in the shell region according to the native blood vessel structure, enabling the emulation of more complex vascular structures with multiple layers and heterogeneous cell populations.

The inherent benefits of coaxial bioprinting involve the precise control of concentric multi-material deposition, one-step facile manufacturing process, flexibility of dimensions (e.g., diameter, wall thickness, and length), and the fabrication of vascular structure with high aspect ratio. However, creating a branched vascular network is difficult owing to the continuous deposition of a uniform tubular structure, which further requires technological convergence and advancements.

2.5. Key elements in 3D bioprinting in *vitro* vascularized models

Complex blood vessels pervade virtually all tissues in the body and affect diverse physiological functions in an organ-specific manner[7]. To better understand how blood vessels develop and function as well as their pathological implications, researchers have strived to engineer 3D living vascular systems that resemble the structure and function of native blood vessels. Innovative approaches to fulfill this critical need have been employed with 3D bioprinting technology. The specific methods of 3D bioprinting applied to vascularization can be categorized into (i) coordinated patterning, (ii) sacrificial, (iii) embedding, and (iv) coaxial bioprinting methods for engineering vascularized tissue models *in vitro*. Specifically, coordinated pattering strategy was mainly used to promote vascular assembly (e.g., vasculogenesis and angiogenesis) for vascular network formation in the bioprinted constructs via endogenous/exogenous induction. Meanwhile, sacrificial, embedding, and coaxial bioprinting strategies were primarily used to create hollow vascular channels within the bioprinted tissue models. Given the functional importance of vasculatures, 3D bioprinting has made transformative progress in reproducing the complex vascular network within engineered tissues.

To establish a functional vascularized model, identifying the key elements to be pursued is crucial. First, the model should include all relevant cell types pertaining to target tissues and should be spatially organized according to the anatomical structures. Second, complex and dynamic ECM environments found in healthy and diseased states of vascularized tissues should be realized to manipulate heterotypic cell–cell communication and cell–ECM interactions. Third, proper biomechanical and biochemical cues must be introduced to orchestrate cellular activities and direct tissue maturity and function. Finally, the model should be easy to reproduce and amenable to commonly used tissue culture and engineering methods, thus facilitating the application of standard evaluation methods of molecular analysis and live imaging. Accordingly, the 3D bioprinting platform can provide fascinating insights into vascularized model development that can not only recreate vascular networks, but also allow biological elements (cells, biomolecules, and their products) to dynamically reorient or remodel an integrated vascular system. This review does not cover the details of cell type, matrix type, and biomaterials used for engineering vasculature, which have been featured elsewhere[6,7,9,13]; however, note that more advanced materials and optimized protocols from cell and molecular biology are necessary for the continued advancement of 3D bioprinting multi-scale functional vasculature. Collectively, the ideal 3D-bioprinted *in vitro* model recapitulates the compositional and structural heterogeneity of the target tissue in living organisms and is amenable to assemble them. We envision that 3D-bioprinted models may provide a much more economical option for advancing the translational research and drug discovery pipeline.

3. Applications of 3D bioprinting in modeling 3D *in vitro* vascular tissues

Because vascularization is a vital component to reconstitute organ-level physiological functions, an emerging direction in establishing a solid vascularized tissue has been pursued to resemble the *in vivo* state within organ-specific vasculature. With advances in 3D bioprinting techniques and biomaterials, 3D *in vitro* models of numerous vital organs and diseases have been developed and validated[6,25,41]. In this section, we discuss recent 3D bioprinting strategies for constructing vascularized tissue models, including specific designs, materials, and fabrication approaches as well as the notable achievements of their applications (Table 2). Here, taking vessel, liver, kidney, and tumor as examples, we review the pioneering and representative work in 3D-bioprinted *in vitro* models.

3.1. 3D bioprinting of perfusable vessel models

Blood vessels are fundamental in circulating nutrients, oxygen, and metabolic wastes and vitalizing most tissues and organs. Given the diffusion limit of living tissues within 200 μm from the nearest capillary, the lack of vasculature in engineering tissues hinders oxygen and nutrient supply and the maintenance of normal physiological conditions. More importantly, manufacturing techniques need to be

Table 2. Summary of 3D-bioprinted vascularized *in vitro* models for different applications

Target	Bioprinting approaches	Biomaterials[a]	Cell types[b]	Major achievements	Ref.
Vessel	Sacrificial	PF-127, gelatin-fibrinogen bioink	HNDFs, HBMSCs, HUVECs	Construction of thick (>1 cm) and long-lasting (>6 weeks) pre-vascularized tissues within customized perfusion chips	[42]
	Coaxial	PF-127, VdECM/alginate hybrid bioink	HUVECs	Creation of a freestanding, perfusable, and functional *in vitro* vascular model with endothelium lining in the luminal wall exhibiting representative vascular functions, including selective permeability, anti-platelets/leukocyte adhesion, and self-remodeling in response to physiological shear stress and directional angiogenesis	[43]
	Embedding + coaxial	PF-127, VdECM/alginate hybrid bioink	HUVECs, HCASMCs, HDFs	Construction of a geometry-tunable triple-layered model of artery equivalent representing recapitulation of hallmarks of early-stage atherosclerosis and dose-dependent drug responses in the platform	[40]
	Coordinated patterning	Fibrinogen-based composite bioink, alginate	HUVECs, HNDFs	Fabrication of an inter-connected, multi-scale micro-vasculature network via spatial gradient of angiogenic factors from the HNDFs, resulting in guided biological self-assembly to direct angiogenic sprouting and micro-vascular networks formation	[44]
Liver	Coordinated patterning	Liver dECM, gelatin	HepaRG, HUVECs	One-step bioprinting of a 3D liver-on-a-chip with vascular/biliary channels representing enhanced liver-specific functions and higher sensitivity of drug responses in the chip compared with simple 2D/3D models	[46]
	Sacrificial	PF-127, GelMA-fibrin bioink	HepG2, HUVECs	Production of a centimeter-scale hepatic tissue with branched perfusable vascular networks, showing improved hepatic marker expressions and high level of albumin secretion	[47]
	Coaxial	Alginate-methylcellulose bioink, collagen-fibrin-gelatin bioink	HepG2, HUVECs, HNDFs	Establishment of *in vitro* triple culture model of hepatic sinusoid-like structure comprising a core compartment with pre-vascular structures and a shell compartment with hepatocytes, revealing improved bioprinted HepG2 functionalities	[48]
	Coaxial	Collagen, alginate, gelatin	HepG2, EA.hy 926	Bioprinting of heterogeneous multi-scale hepatic lobules with a pre-set arrangement of hepatocytes and endothelial cells with a central lumen, which can preserve structural integrity and promote hepatic maturation	[49]
	Coordinate patterning	Liver dECM, gelatin	HepG2, LX-2, HUVECs	3D bioprinting of liver fibrosis-on-a-chip with organized biomimetic hepatic layers and cellular distribution under liver fibrosis conditions, exhibiting hallmarks of fibrotic remodeling mainly due to the presence of the stellate cell population	[50]
Kidney	Sacrificial	PF-127, gelatin-fibrin bioink	PTECs	Development of a convoluted renal proximal tubule model on perfusable chip displaying the formation of a tissue-like polarized epithelium with improved phenotypes and superior albumin uptake function	[28]
	Sacrificial	PF-127/PEO ink, gelatin-fibrin bioink	PTECs, GMECs	Engineering a 3D vascularized proximal tubule model with co-localized vascular and proximal tubular channels for *in vitro* studies of kidney function, disease modeling, and pharmacology	[53]
	Coaxial	PF-127, kidney dECM/alginate hybrid bioink	RPTECs, HUVECs	Construction of vascularized kidney model with tubular/vascular hollow channels with improved renal functionalities of the epithelia barrier	[54]
	Coaxial	PF-127, kidney dECM/alginate hybrid bioink	Caco-2, HK-2, GEs	Construction of a perfusable *in vitro* multi-organ model resembling pathophysiological features of secondary hyperoxaluria, including glucose reabsorption and tubular fluid flow behavior-dependent CaOx crystal formation	[55]

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Tumor	Coordinated patterning	Brain dECM bioink	Patient-derived GBM cells, HUVECs	Development of a patient-specific GBM-on-a-chip with compartmentalized concentric-ring structure emulating key pathological features of GBM, which demonstrated identification of patient-specific responses to chemoradiotherapy	[59]
	Sacrificial	PF-127, fibrin bioink	Patient-derived GBM cells, hAstro, hMG, hPericytes, HUVECs	Engineering a complex GBM model consisting of tumor/stroma compartment and perfusable vascular channel to establish the penta-culture system to simulate GBM cellular heterogeneity, cell–cell interaction, and spatial topology	[60]
	Sacrificial	Gelatin, collagen	Patient-derived GBM cells, HUVECs	3D bioprinting of GBM models with perfusable vascular channels and patient-derived GBM spheroids allowing GBM invasion and long-term (up to 70 days) culture/drug delivery	[61]
	Coordinated patterning	Fibrin	A549, HUVECs, HDFs	Production of a spatially-defined 3D *in vitro* metastatic model via spatiotemporal control of signaling molecular gradients to mimic key steps of cancer dissemination	[62]
	Embedding + coaxial	PF-127, Skin dECM, VdECM/alginate hybrid bioink	SK-MEL-28, HUVECs	Construction of a tissue level cancer-vascular platform for modeling metastatic melanoma with highly controllable cancer-vascular interactions, investigating metastasis-relevant changes in the platform	[63]
	Coaxial	PEGDA, PEGOA, GelMA, alginate	MCF-7, HUVECs	Development of a breast cancer-on-a-chip with the pair of perfusable blood vessels and hollow lymph vessel with one end-blinded to investigate doxorubicin delivery profiles	[65]
	Embedding + coaxial	PF-127, Skin dECM, VdECM/alginate hybrid bioink	SK-MEL-28, HNDFs, HDMECs, HDLECs,	3D bioprinting of a perfusable blood-lymphatic integrated system with melanoma heterospheroids, which replicated hallmark events of metastatic melanoma (tumor stroma interaction, melanoma invasion, and intravasation) and evaluated anti-metastatic effect of combinational targeted therapy	[66]

[a]Abbreviations of biomaterials—Pluronic F127: PF-127, VdECM: vessel tissue-derived decellularized extracellular matrix, fibrinogen-based composite bioink composed of fibrinogen, gelatin, hyaluronic acid, and/or alginate, GelMA: gelatin methacryloyl, PEO: poly(ethylene oxide), PEGDA: Polyethylene glycol diacrylate, PEGOA: 8-arm polyethylene glycol-octaacrylate.
[b]Abbreviations of cell types—HNDFs: human neonatal dermal fibroblasts, HDFs: human dermal fibroblasts, HBMSCs: human bone marrow mesenchymal stem cells, HUVECs: human umbilical vein endothelial cells, HCASMCs: human coronary artery smooth muscle cells, PTECs: human proximal tubule epithelial cells, GMECs: glomerular micro-vascular endothelial cells, RPTECs: renal proximal tubule epithelial cells, GEs: human glomerular endothelial cells, GBM: glioblastoma, hAstro: human astrocytes, hMG: human microglia, hPericytes: human micro-vascular brain pericytes, HDMECs: human dermal micro-vascular endothelial cells, HDLECs: human dermal lymphatic endothelial cells.

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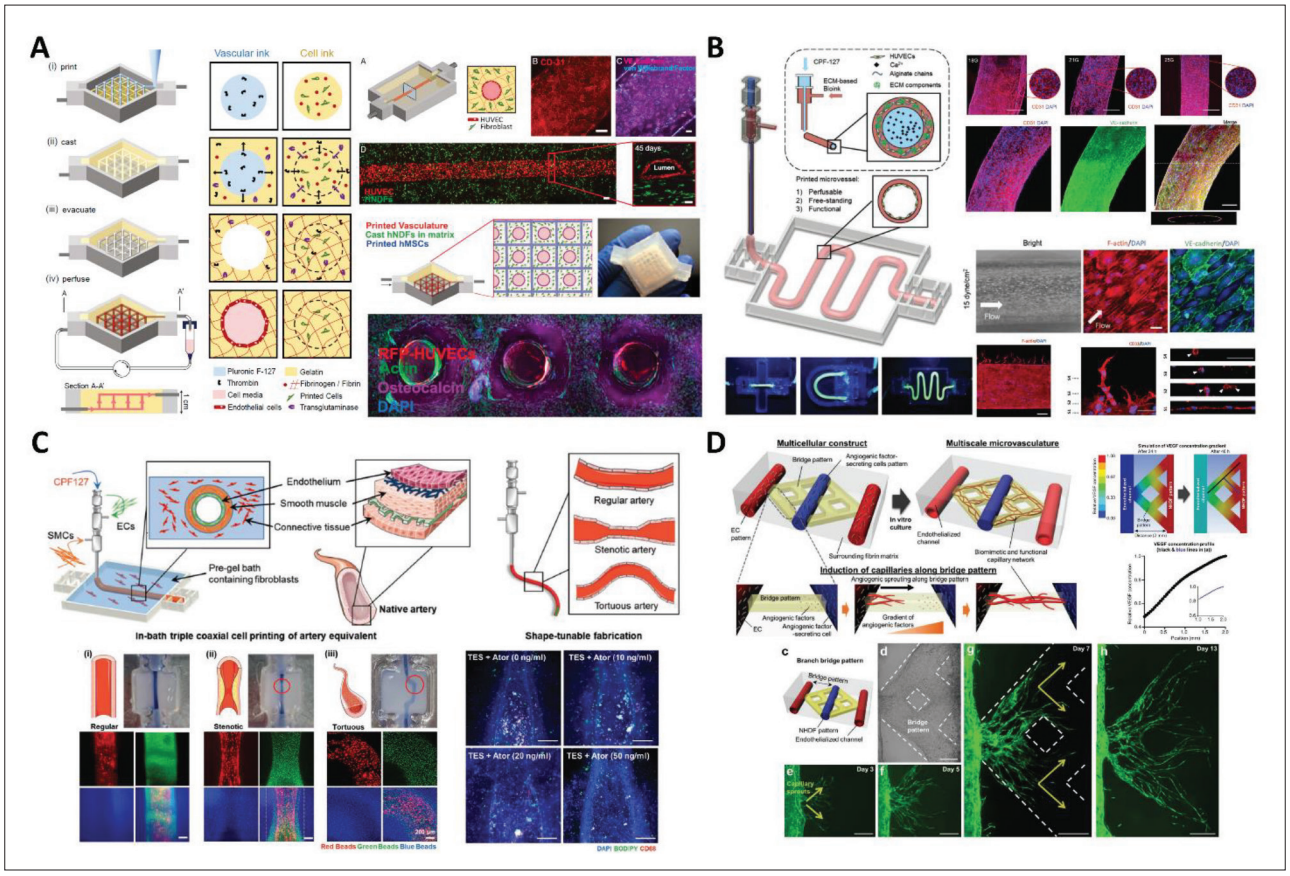

Figure 1. 3D bioprinting of perfusable vessel models. (A) Sacrificial bioprinting for the construction of a thick and a thick (>1 cm) and long-lasting (>6 weeks) pre-vascularized tissues within customized perfusion chips[42]. (B) Coaxial bioprinting of a freestanding, perfusable, and functional *in vitro* vascular model with endothelium lining in the luminal wall. Reproduce with permission from Gao G, Park JY, Kim BS, *et al., Adv Healthc Mater,* 2018, Copyright © 1999-2023 John Wiley & Sons[43]. (C)Construction of a geometry-tunable triple-layered model of artery equivalent using coaxial and embedding bioprinting strategies. Reproduce with permission from Gao G, Park W, Kim BS, *et al., Adv Funct Mater*, 2021, Copyright © 1999-2023, John Wiley & Sons[40]. (D) Coordinated patterning for fabricating an inter-connected, multi-scale micro-vasculature network with spatial gradient of angiogenic factors. Reproduced with permission from Son J, Hong SJ, Lim JW, *et al., Small Methods*, 2021, Copyright © 1999-2023, John Wiley & Sons[44].

developed to fabricate perfusable vascular networks and thus achieve vascular functions. Many approaches have been proposed to mimic vascular network architecture and composition. Among them, 3D bioprinting inherently helps to generate functional and reproducible vascular networks that can model 3D vascular structures at a cellular scale with luminal perfusion. Kolesky *et al.*[42] demonstrated the feasibility of fabricating thick (>1 cm) and long-lasting (>6 weeks) pre-vascularized tissues within customized perfusion chips using the sacrificial bioprinting method (Figure 1A). The sacrificial ink of PF-127 solution containing thrombin was used to print macro-channels with diameters of approximately 200–300 μm, followed by the casting of the ECM material, including gelatin, fibrinogen, transglutaminase, and cells (human neonatal dermal fibroblasts and human bone marrow-derived mesenchymal stem cells), over the printed fugitive inks. Following the removal of PF-127, the thick vascularized model was reproduced by introducing ECs into a perfusable micro-channel, resulting in the inter-connected vascular formation that supported endothelialization and retained cell viability over 95%. Another strategy, coaxial bioprinting, was introduced by Gao *et al.*[43] to establish a freestanding, perfusable, and functional *in vitro* vascular model using a core/shell nozzle and a hybrid bioink comprising vascular tissue-specific bioink and alginate (Figure 1B). This one-step fabrication approach allowed the creation of diverse vascular structures with endothelium lining in the luminal wall. Upon cultivation, the model revealed representative vascular functions, including selective permeability, anti-platelets/leukocyte adhesion, self-remodeling in response to physiological shear stress, and directional angiogenesis. This coaxial bioprinting approach was further extended to create a triple-layered vascular model composed of endothelium, smooth muscle, and connective tissue, which more closely resembled the native blood vessel structures.

As a combinatorial approach using coaxial and embedding bioprinting methods, Gao *et al.*[40] described 3D in-bath coaxial cell printing with vascular-specific ECM bioinks to mimic the regular straight, stenotic, and tortuous models of arterial constructs (Figure 1C). They designed a customized triple coaxial nozzle composed of PF-127/$CaCl_2$ solution in the core nozzle, human umbilical vein endothelial cells (HUVECs)-laden VdECM/alginate hybrid bioink in the middle nozzle, and human coronary artery smooth muscle cells-laden VdECM/sodium alginate bioink in the outer nozzle. Several triple-layer artery equivalents with tunable geometries (e.g., regular straight, stenotic, and tortuous models) were fabricated by tuning the printing bath and moving speed. In addition, the proposed triple-layer model could recapitulate the hallmark events in early-stage atherosclerosis, such as endothelial activation, macrophage adhesion and differentiation, low-density lipoprotein accumulation, and foam cell formation. Furthermore, the developed model was implemented to evaluate the dose-dependent effect of atorvastatin on the suppression of foam cell formation, thus highlighting the advantages of the in-bath coaxial bioprinting approach and its potential for a drug screening platform. Despite significant efforts in creating readily perfusable vascular channels, there is still a lack in the production of micro-vascular networks owing to the limitation of the extrusion-based printing resolution. To circumvent this, Son *et al.*[44] interestingly proposed a micro-vascular induction strategy that introduced angiogenic factor-secreting cells—that is, normal human dermal fibroblasts (NHDFs)—to create angiogenic factor gradients along a bridge pattern (Figure 1D). With a coordinate pattering approach, a multi-cellular construct composed of EC patterns, an angiogenic factor-secreting cell pattern, bridge patterns, and a surrounding fibrin matrix was designed to produce a functional, multi-scale micro-vasculature with tissue-specific capillary networks. Following this method, the spatial gradient of angiogenic factors secreted from the NHDFs resulted in inducing biological self-assembly to direct angiogenic sprouting and micro-vascular networks formation. The study evaluated the morphological and functional connectivity between endothelialized channels and capillary networks, which may have potential in the fabrication of high-density and organotypic multi-scale micro-vasculature.

Various bioprinting approaches have been used alone or in combination to fabricate a perfusable micro-channel with endothelialized networks, allowing improved vascular function and maturation in an integrated perfusion platform. We envision adopting 3D bioprinting that offers a promising avenue for the generation of human organ-specific vascularization with excellent structural complexity and physiologically relevant levels of function.

3.2. 3D bioprinting of vascularized liver models

The liver is the largest solid organ in the human body and performs multiple physiological functions, including metabolism, detoxification, bile production, and filtration. The liver constitutes complex hepatic lobules that are tightly assembled into a 3D hexagonal structure. This distinct micro-structural organization confers the multi-cellular communication primarily responsible for hepatic function[45]. A compelling need has emerged to develop a liver model that replicates the morphological and biological complexities of the liver for tissue development, disease modeling, and drug screening applications. Recent advances in 3D bioprinting encourage the development of a 3D biomimetic hepatic *in vitro* model that more precisely emulates the complex microenvironment of the liver. Using 3D bioprinting, several liver tissue models have been constructed to achieve liver functions *in vitro* for different research objectives. Lee *et al.*[46] proposed a one-step 3D bioprinting approach to design and fabricate a 3D liver-on-a-chip platform introducing a co-culture of multiple cell types (Figure 2A). The housing and micro-fluidic channels of the chip were built using poly(ethylene/vinyl acetate) (PEVA), while the heterotypic cell-laden bioinks were precisely placed in the desired location within chip frame. Furthermore, HepaRG-laden liver dECM and HUVEC-laden gelatin bioinks were printed into the two fluidic channels, respectively (a vascular channel on top and a biliary channel bottom), resulting in a bilayer structure to simulate the liver-biliary duct system that is critical for bile acid excretion. A 3D liver-on-a-chip with vascular/biliary fluidic channels induced better biliary formation and improved liver-specific gene expression and hepatic function when compared to a chip without a biliary system. Further, the chip was assessed using acetaminophen, and the results showed a more sensitive drug response in the chip than in the 2D culture condition, highlighting the feasibility of 3D-bioprinted liver-on-a-chip to investigate drug metabolism and toxicity. Another study by Liu *et al.*[47] utilized sacrificial printing to create centimeter-scale liver-like tissues using cell-laden GelMA-fibrin ink and fugitive PF-127 ink (Figure 2B). A 3D-bioprinted hepatic tissue with branched perfusable vascular networks was obtained by endothelializing the printed macro-scale channels along with capillary networks through cell self-assembly, showing enhanced hepatic marker expressions and higher levels of albumin secretion. Taymour *et al.*[48] recently employed coaxial bioprinting to develop a liver sinusoid-like model composed of a core compartment with pre-vascular structures and a shell compartment with hepatocytes (Figure 2C). For the core part, gelatin was added to a natural ECM-like core ink based on collagen and fibrinogen with human ECs and fibroblasts to form a stable pre-vascular network. The shell part was based on

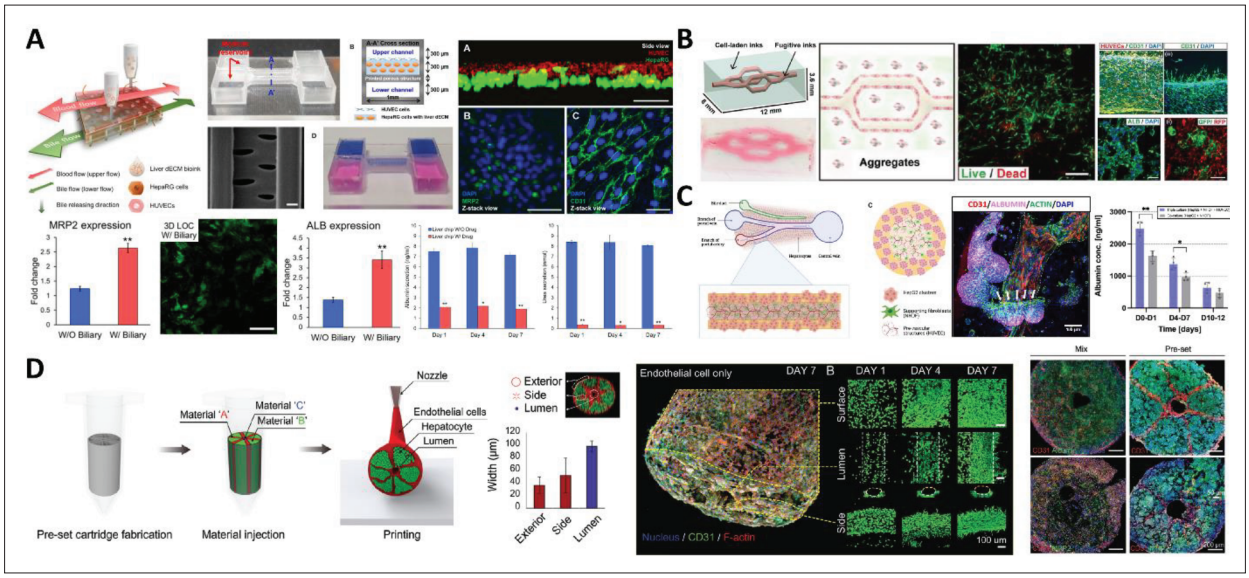

Figure 2. 3D bioprinting of vascularized liver models. (A) Coordinated patterning strategy to fabricate a 3D liver-on-a-chip with vascular/biliary channels in a one-step process. Used with permission of Copyright 2023 IOP Publishing, from Cell-printed 3D liver-on-a-chip possessing a liver microenvironment and biliary system, Lee H, Chae S, Kim JY, *et al*, 11(2): 025001, 2019; permission conveyed through Copyright Clearance Center, Inc[46]. (B) Sacrificial bioprinting strategy to produce a centimeter-scale hepatic tissue with branched perfusable vascular networks. Reproduced with permission from Liu X, Wang X, Zhang L, *et al., Adv Healthc Mater*, Copyright © 1999-2023, John Wiley & Sons[47]. (C) Coaxial bioprinting strategy to establish in vitro triple culture model of hepatic sinusoid-like structure comprising a core compartment with pre-vascular structures and a shell compartment with hepatocytes (from ref.[48] licensed under Creative Commons Attribution 4.0 license). (D) Advanced coaxial bioprinting strategy to generate heterogeneous multi-scale hepatic lobules with a pre-set arrangement of hepatocytes and endothelial cells with a central lumen. Images reproduced with permission from Kang D, Hong G, An S, *et al., Small*, Copyright © 1999-2023, John Wiley & Sons[49].

alginate and methylcellulose dissolved in human fresh frozen plasma that endowed the core–shell constructs with excellent printability and supportive bioactivity. Thus, core–shell printing allowed for establishing an *in vitro* triple culture model of the hepatic sinusoid to investigate cell behavior in response to tailoring the 3D microenvironment. Interactions between the different cell types in the triple culture model were characterized in terms of the bioprinted HepG2 functionality (i.e., improved albumin secretion in the presence of HUVEC, the necessity of supportive fibroblasts for angiogenesis, and the competing effect of HepG2). In a different study, Kang *et al.*[49] proposed the pre-set extrusion bioprinting in which different bioinks were simultaneously extruded through a precursor cartridge to create multi-material patterning according to the pre-defined configuration without structural deformation (Figure 2D). Using this approach, a heterogeneous, multi-cellular, and multi-material construct was bioprinted to form a hepatic lobule-like structure with a pre-set arrangement of hepatocytes and ECs with a central lumen. The liver construct with biomimetic heterocellular localization was found to enhance hepatic maturation compared with mix-printed constructs lacking geometric structure, as evidenced by the enriched secretion of urea and albumin, protein levels of albumin, MRP2, and CD31, and cytochrome P450 enzyme activity; this emphasizes the importance of architectural cellular positioning to promote hepatic and vascular function. In addition to physiological liver tissue modeling, 3D-bioprinted liver tissue can be applied in disease modeling. For example, to develop a liver fibrosis model, coordinated patterning was used to generate organized hepatic layers composed of hepatocytes, activated stellate cells, and ECs[50]. Through the multi-material printing process, each non-parenchymal hepatic cell type was accurately localized into a multi-layered construct using gelatin and liver dECM bioinks. While hepatocytes-laden liver dECM bioink was exploited to provide a 3D liver-specific microenvironment, gelatin bioink was used to deliver ECs or activated stellate cells for monolayer formation. Overall, a unique multi-layered liver fibrosis model was reproduced to mimic cellular distribution of human liver fibrosis. The 3D-bioprinted liver fibrosis-on-a-chip platform exhibited hallmarks of fibrotic remodeling, including collagen accumulation, cell apoptosis, and reduced liver function, mainly owing to the presence of the stellate cell population. For drug screening application, drugs for downregulating hepatic stellate cell activation were treated on the 3D-bioprinted liver fibrosis

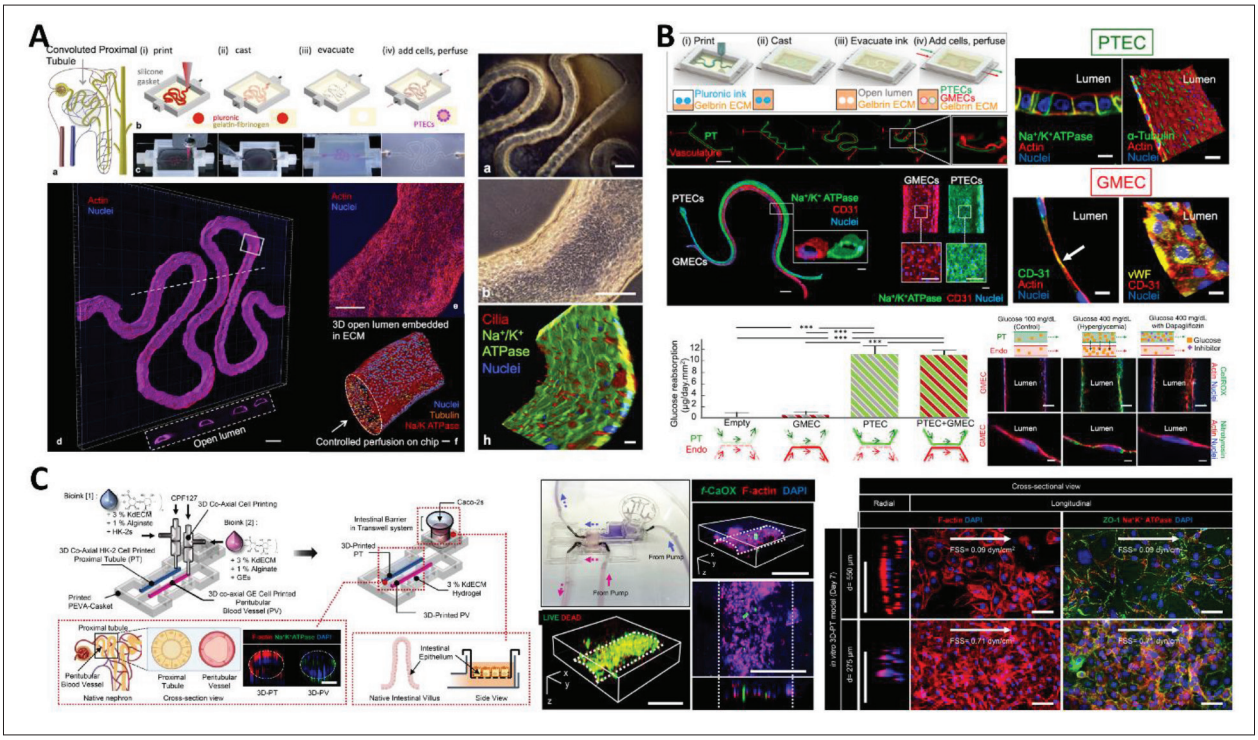

Figure 3. 3D bioprinting of vascularized kidney models. (A) Sacrificial bioprinting for creating a 3D convoluted renal proximal tubule model on customized perfusable chip [from ref.[28] licensed under Creative Commons Attribution 4.0 license]. (B) Sacrificial bioprinting for engineering a 3D vascularized proximal tubule model with co-localized vascular and proximal tubular channels [from ref.[53] licensed under Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND)]. (C) Coaxial bioprinting of a perfusable in vitro multi-organ model resembling pathophysiological features of secondary hyperoxaluria. Reprinted from Yoon J, Singh NK, Jang J, *et al.*, 2022, 3D bioprinted in vitro secondary hyperoxaluria model by mimicking intestinal-oxalate-malabsorption-related kidney stone disease. *Appl Phys Rev*, 9(4): 041408 , with the permission of AIP Publishing[55].

model, which facilitated in the evaluation of an anti-fibrogenic effect of drug combination.

In sum, current liver *in vitro* models recapitulate the structural and functional features of hepatic lobules and vascular networks to certain extent. Despite successful attempts, challenges still remain in the fully reproducing the liver functions *in vitro* through the 3D bioprinting of vascularized liver models. Owing to the natural heterogeneity of the liver composition and architecture, the synchronous control of microenvironmental cues, including signaling pathways, heterocellular organization, metabolism, ECM, and fluidics, for hepatic and vascular development is key to modeling a fully functional liver. Robust vascular systems with ample multi-scale vasculature also need to be addressed to ensure the long-term investigation of bioprinted liver models.

3.3. 3D bioprinting of vascularized kidney models

The kidney is responsible for the maintenance of body fluids homeostasis and waste excretion functions. Nephron is a complex functional unit of the kidney with the ability to filter and reabsorb solutes from the bloodstream, and these functions significantly rely on the active interaction between renal tubules and vascular networks. Most kidney models are based on micro-fluidic chip devices that simulate fluid environment to support renal tubular cell growth and function[51,52]. However, their anatomical structures, complexity, and functionality are still lacking to fully model renal tissue. To recapitulate the structural and functional complexity of the kidney, 3D bioprinting provides an attractive strategy for developing kidney *in vitro* models with tube-like micro-fluidic structures[28,53,54]. Homan *et al.*[28] developed a convoluted renal PT model on perfusable chips (Figure 3A). The sacrificial bioprinting approach was employed to prepare a perfusable channel using fugitive sacrificial PF-127 and a gelatin-fibrin ECM hydrogel. After the removal of PF-127, human proximal tubule epithelial cells (PTECs) were introduced within the hollow channel to form a 3D tubule with epithelial lining. Compared with PTEC 2D models, the perfusable 3D PT model exhibited the formation of a tissue-like polarized epithelium with improved phenotypes and superior albumin uptake function. The mature epithelium was applied in testing nephrotoxicity with a known compound (i.e., cyclosporine A). Upon exposure,

the epithelial barrier disrupted in a dose-dependent manner. This sacrificial approach was further expanded by Lin *et al.*[53] to produce vascular and proximal tubular channels circumscribed by proximal tubular epithelium and kidney endothelium within a gelatin-fibrin gel, displaying co-localized lumens with a separation distance of approximately 70 μm. Continuous flow through the channels was controlled using a closed-loop perfusion system in a 3D vascularized PT model to study the renal reabsorption of solutes, which showed active reabsorption via tubular–vascular exchange (Figure 3B). Moreover, hyperglycemia-induced EC dysfunction was replicated in the vascularized PT model, and the effect of a glucose transport inhibitor was investigated in hyperglycemic disease conditions. To directly fabricate tubular structures, Singh *et al.*[54] described a coaxial bioprinting strategy for fabricating micro-fluidic tubes mimicking tubular/vascular renal parenchyma comprising renal tubular epithelial cells and ECs. With the aid of kidney-derived ECM bioink, this 3D coaxially-bioprinted vascularized renal PT model replicated the micro-physiological environment, exhibiting improved renal functionalities of the epithelial barrier akin to native renal tubular tissue. Thus, the combination of tissue-specific bioactive inks and coaxial bioprinting of the renal tubules can generate functional kidney units. The same research group expanded their coaxial strategy for the disease modeling of secondary hyperoxaluria to resemble oxalate malabsorption-related intestinal epithelium and kidney stone formation. Recently, Yoon *et al.*[55] introduced an integrative approach to construct a perfusable *in vitro* multi-organ model mimicking the key pathophysiological features of secondary hyperoxaluria (Figure 3C). To develop a multi-organ model, coaxial bioprinting was used to spatially compartmentalize intestinal epithelium and a vascularized PT. The model exhibited several biophysical features, including glucose reabsorption and tubular fluid flow behavior-dependent CaOx crystal formation. The features were attributable to the establishment of fluidically inter-connected multi-organ modules, and the model allowed to dissolve CaOx crystal following the perfusion of trisodium citrate and grape seed extract.

Collectively, 3D bioprinting-assisted kidney models provide an *in vitro* experimental platform for investigating kidney function, disease modeling, and drug testing. The developed perfusable kidney *in vitro* models showed a notable improvement in enriched vasculature and promoted maturity and function within their renal analogs. However, despite the promising outcomes together with multiple 3D bioprinting strategies, key cell populations, tissue-specific ECM compositions, and multi-scale structural complexity should be considered for further advancing complex kidney *in vitro* model development.

3.4. 3D bioprinting of vascularized tumor models

Cancer is a multifaceted pathology entailing inherently complex structures and heterogeneous cell populations. To comprehensive understand sophisticated diseases such as cancer, 3D tumor models have emerged as a powerful tool in cancer research and drug screening. In tumor modeling, complex tumor–stroma interaction is a key signature of most malignant tumors that ushers cancer progression, metastasis, and drug resistance, ultimately resulting in treatment failure[56]. Research has paid much attention toward precisely modeling the complexity and dynamic interactions of the tumor microenvironment (TME). However, conventional tumor models are known to be sub-optimal in realizing human cancer physiology, which imposes tremendous constraints on the anti-cancer drug efficacy[57,58]. Thus, a pressing need exists to develop physiologically relevant 3D cancer models that can reproduce the complexity of the TME, including stroma–immune interactions, angiogenesis, and ECM remodeling. In this context, 3D bioprinting, with its ability to create highly controlled complex 3D culture systems, provides a competitive advantage over other biofabrication methodologies. To date, bioprinted vascularized tumor models have drawn considerable attraction in the depiction of tumorigenesis, tumor angiogenesis, tumor metastasis, and tumor interactions. Yi *et al.*[59] presented a model of the glioblastoma microenvironment (GBM) through spatial deposition of patient-derived GBM cells and ECs with brain dECM bioink. Using coordinated pattering strategy, GBM-on-a-chip was reconstructed in a compartmentalized cancer–stroma concentric-ring structure to capture the key environmental properties of GBM, such as central hypoxia with a radial oxygen gradient, and a heterogeneous ECM microenvironment. Importantly, the GBM-on-a-chip incorporating patient-derived cells exhibited clinically observed patient-specific treatment resistances to concurrent chemoradiation and temozolomide drug. Such *ex vivo* cancer chip platform is helpful for identifying clinically effective therapies and determining effective drug combinations over extremely lethal brain cancer such as GBM. Neufeld *et al.*[60] developed an intricately perfusable glioblastoma tumor model comprising two compartments of tumor/stroma and blood vessels (Figure 4A). The major tumor/stroma compartment was bioprinted using a fibrin bioink containing patient-derived glioblastoma cells, astrocytes, and microglia. Perfusable blood vessels were sacrificially bioprinted along with a customized pattern to resemble a 3D lumen vascular structure using a fugitive PF-127 ink and subsequently lined with brain pericytes and ECs. In their study, the penta-culture system simulated GBM cellular heterogeneity, cell–cell interaction, and spatial topology. Patient-derived GBM cells cultured in the 3D-bioprinted

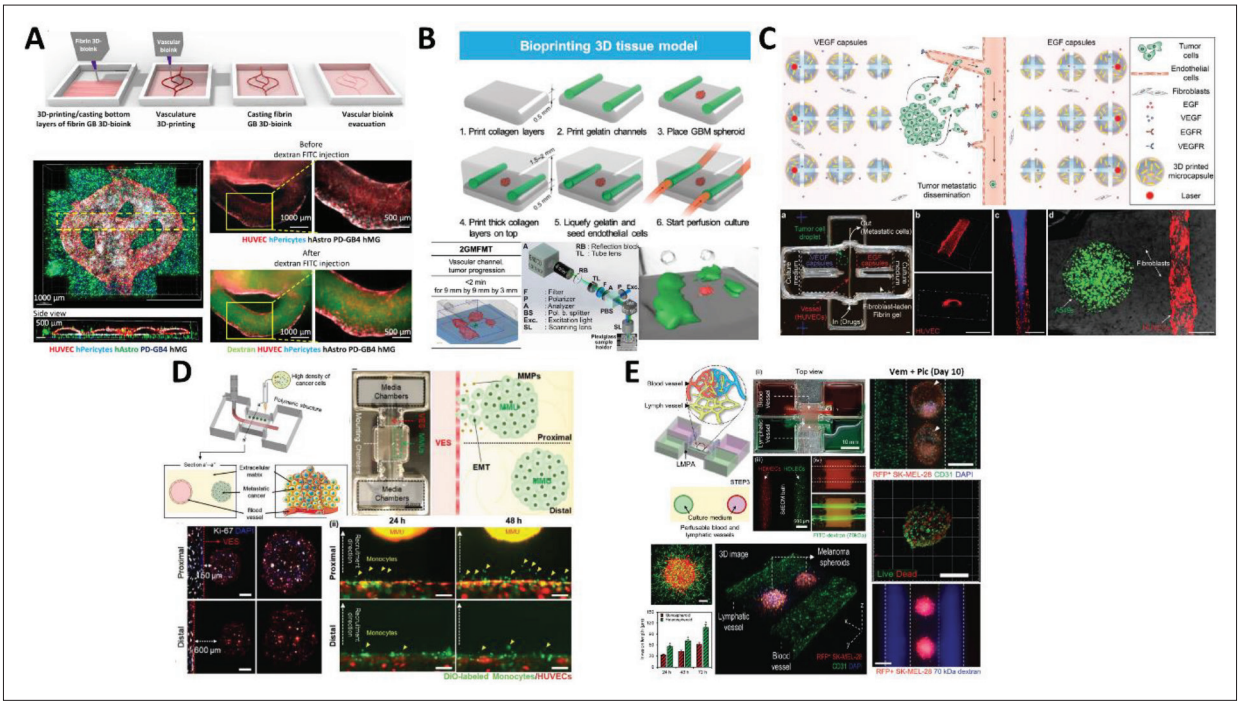

Figure 4. 3D bioprinting of vascularized tumor models. (A) Sacrificial bioprinting strategy to engineer a complex GBM model consisting of tumor/stroma compartment and perfusable vascular channel in the penta-culture system. (B) Sacrificial bioprinting strategy to establish a biomimetic GBM model combining perfusable vascular channels and patient-derived GBM spheroids. Figure 4A and 4B from ref. (60) licensed under Creative Commons Attribution NonCommercial License 4.0 (CC BY-NC). (C) Coordinated patterning approach to produce a spatially-defined 3D in vitro metastatic model. Reproduced with permission from Meng F, Meyer CM, Joung D, *et al., Adv Mater,* Copyright © 1999-2023 John Wiley & Sons [62]. (D) A combination of embedding and coaxial bioprinting strategies to construct a tissue-level cancer-vascular platform composed of a metastatic cancer unit and a perfusable vascular system and to develop an advanced blood-lymphatic integrated system with melanoma heterospheroids. Reproduced with permission from Cao X, Ashfaq R, Cheng F, *et al., Adv Funct Mater,* Copyright © 1999-2023 John Wiley & Sons. (E) Both for modeling metastatic melanoma from ref.[66] licensed under Creative Commons Attribution 4.0 International (CC BY 4.0).

models exhibited several tumorigenic characteristics (i.e., growth kinetics, drug response, and genetic signature) that were more similar to the murine *in vivo* tumor setting than the 2D culture model. 3D-bioprinted *in vitro* cancer models with increased complexities in structure and biology of GBMs may likely serve as a rapid and personalized drug screening platform. For drug delivery studies, Ozturk *et al.*[61] developed a 3D-bioprinted GBM model combining perfusable vascular channels using sacrificial bioprinting (Figure 4B). To establish the GBM model, the two gelatin channels were integrated into the collagen layers, followed by liquefying the gelatin and seeding the ECs into fluidic channels to form the lumen structure. Meanwhile, patient-derived GBM spheroids (>400 μm in diameter) were placed between the vascular channels, permitting GBM invasion and an extended life span of the tumor for up to 70 days. Following temozolomide treatment, GBM spheroids revealed regressing tendency; however, some GBM cells survived and resumed their active invasion despite continued drug treatment, implying long-term therapeutic resistance. In particular, their novel 3D imaging modality enabled the efficient, long-term, non-invasive imaging of 3D-bioprinted tumor constructs for the volumetric alteration of tumor mass and drug response assessment.

Metastasis is the process by which cancer cells spread from a primary site to other parts of the body moving through the vascular system. Thus, establishing a vascular network plays a crucial role in modeling metastasis progression. Meng *et al.*[62] created a spatially-defined 3D *in vitro* metastatic model via 3D bioprinting where the tumor cell droplet as the primary tumor and endothelialized micro-channels were assembled within the fibroblast-laden fibrin matrix as tumor stroma to mimic the key aspects of metastasis, including invasion, intravasation, and angiogenesis (Figure 4C). Furthermore, a spatiotemporal gradient of growth factors (e.g., epidermal growth factor and vascular endothelial growth factor) was achieved using programmable release capsules (triggered by laser irradiation) to reproduce the biochemical features of the TME. In the emulated metastasis model, lung cancer cell invasion and intravasation into the engineered vasculature were observed under the guidance of signaling molecule gradients. The study confirmed the suitability

of this bioprinted metastasis model for drug screening applications by validating the anti-cancer efficacy of the immunotoxin. In another study by Kim *et al.*[63], a cancer-vascular platform involving a metastatic cancer unit and a perfusable vascular system was constructed via the embedding and coaxial bioprinting techniques (Figure 4D). Using the former, size-tunable 3D tumor spheroids were generated with a high density of melanoma cells in the suspension bath matrix of skin dECM, mimicking the *in vivo* scenario (e.g., local invasion, central hypoxia, and angiogenic signaling) of metastatic cancer. Through coaxial bioprinting, a vessel-like tubular structure was fabricated using HUVECs-laden VdECM/alginate hybrid bioink within skin dECM bath. Thus, 3D tumor spheroids with 600 μm diameter and perfusable vascular channel were directly printed within tissue-specific bath ink with high-precision positioning control in a single step. Such tissue-level cancer-vascular platforms allowing distance control were evaluated to investigate whether positional changes influence cancer progression and metastasis, such as epithelial–mesenchymal transition, endothelial dysfunction, angiogenic sprouting, and monocytes recruitment, corroborating the positional importance in tumor metastasis. This unique bioprinting approach will likely provide a reliable platform that can simulate metastatic cancer progression in a more realistic status *in vitro*.

In addition to engineering perfusable vasculature, integrating lymphatic vessels is of a critical aspect to model cancer metastasis. Vascular-lymphatic circulation systems offer a specialized recycling circuit to the most administered anti-cancer drugs *in vivo*[64]. Cao *et al.*[65] proposed a breast-cancer-on-a chip platform comprising a hollow blood vessel and a lymphatic vessel pair using coaxial bioprinting. The pairs of perfusable blood vessel and hollow lymph vessel with one end blinded were fabricated using polyethylene glycol diacrylate/alginate/GelMA and eight-arm poly (ethylene glycol) acrylate/alginate/GelMA inks, respectively, with tunable diffusion properties imitating their native counterparts. Then, the printed tubes and MCF-7 breast cancer cells were embedded in the GelMA matrix to conduct anti-cancer drug (doxorubicin; DOX) delivery. Using this platform, DOX transport profiles were investigated with regard to different combinations of the blood and lymphatic vessels and tumor cell arrangements. More recently, Cho *et al.*[66] constructed a more advanced blood-lymphatic integrated system with heterospheroids by employing a combination of embedding and coaxial bioprinting strategies (Figure 4E). Taking metastatic melanoma as example, a 3D-bioprinted perfusable blood-lymphatic integrated system with heterogeneous spheroids was developed to better recapitulate metastatic melanoma TME. They extended their elegant approach of in-bath coaxial bioprinting, as reported in previous work[63]. The size-controllable multi-cellular metastatic melanoma spheroids with a perfusable blood and lymphatic vessel pair were systematically integrated to develop a 3D *in vitro* melanoma model with improved emulation of the complex TME. The system replicated hallmark events of metastatic melanoma, such as tumor–stroma interaction, melanoma invasion, and intravasation. In addition, the anti-metastatic effect of combinational targeted therapy was evaluated by monitoring drug responses based on the interaction between melanoma heterospheroids and the surrounding TME.

Taken together, 3D bioprinting is a promising approach to generate a reproducible and robust 3D vascularized tumor model through the elaboration of multi-cellular constructs within a controlled spatial environment. Ultimately, personalized drug screening using 3D-bioprinted cancer models containing patient-derived cells provides an exciting opportunity to navigate clinical decision for identifying optimal treatment regimens specific to individuals. However, all these bioprinted platforms still have challenges to be overcome, including their level of high-throughput production capability and poor cellular heterogeneity with genomic stability.

3.5. 3D Bioprinting of other vascularized tissue models

3D bioprinting has helped spur the development of several tissue/organ models for *in vitro* studies. Other vascularized *in vitro* tissue models of great interest include bone and skin. Few studies have demonstrated their feasibility in reproducing intricate geometry and representative physiological functions of the target tissues.

Considering bone tissue models, 3D bioprinting takes advantage of the combination of functional vascular and osteogenic components within the construct for vascularized bone tissue formation *in vitro*. For example, Chiesa *et al.*[67] reported the *in vitro* construction of bone tissue using a gelatin-nanohydroxyapatite (Gel-nHAp), human mesenchymal stem cells (hMSCs), and HUVECs. Based on a coordinate pattering approach, bone construct with an inter-connected pore network was first created using Gel-nHAp followed by hMSCs being seeded on scaffolds and osteogenically differentiated for 2 weeks. Then, a suspension containing a 4:1 ratio of hMSCs and HUVECs entrapped in fibrin-GelMA gel was placed in the macro-pores of the 3D-bioprinted scaffold to induce angiogenesis for 2 weeks. This approach resulted in developing a self-assembly-driven *in vitro* vascularized bone model, confirming *de novo* morphogenesis of capillary-like networks, vascular lumen formation, and

osteogenesis. As another application, the skin has been a major focus of cosmetic research as it performs many essential physiological functions including protection, conditioning, and sensation. Faced with the restrictions on animal testing for cosmetic products, engineering 3D skin model provides a valuable alternative to animal models. 3D bioprinting bolsters development of vascularized human skin equivalents as a novel *in vitro* skin model for drug or cosmetic testing platform. Kim *et al.*[68] constructed a perfusable and vascularized 3D skin equivalent mimicking the structural complexity of the human skin. In the custom-made polycaprolactone transwell chambers, the bioprinted full-thickness skin models consisted of an epidermis (primary human epidermal keratinocytes), dermis (human dermal fibroblast-loaded skin dECM with fibrinogen), and hypodermis (preadipocyte-laden adipose dECM with fibrinogen). Gelatin and thrombin (sacrificial material) loaded with HUVECs was utilized to form vascular channels. They demonstrated that the engineered full-thickness skin models replicated the structural complexity of native human skin more realistically compared to the conventional dermal and epidermal skin models. Moreover, the vascularized dermal and hypodermal compartments promoted interactions with the epidermal compartment, emulating native-like epidermal morphogenesis. The unique 3D-bioprinted platform of a perfusable vascularized human skin equivalents exhibited tissue development and closely mimicked natural human skin. Therefore, 3D bioprinting has provided a viable solution to the development of *in vitro* vascularized models of various tissues for interrogating human pathophysiology and pre-screening drug candidates.

4. Conclusion and future outlook

An unrelenting pressure exists to ascertain alternatives to traditional pre-clinical models, such as simple cell culture or animal testing, for better replicating biological or therapeutic responses detected in humans[5,69]. This has led to the emergence of 3D *in vitro* models owing to their ability to faithfully recapitulate the key architectural and physiological characteristics within an *in vitro* setting. Such models are expected to serve as reliable tools to narrow the gap between oversimplified planar culture systems and species-discrepant animal models. Note that vascularization is of utmost importance for the supply of oxygen and nutrients and for the manipulation of communication between cells and their extracellular environment. Therefore, engineering biomimetic and multi-scale vascular networks is essential for establishing physiologically relevant 3D *in vitro* models.

As cutting-edge biofabrication technology, 3D bioprinting enables the production of 3D living constructs in desired complexity and arrangement, which holds tremendous promise to tackle multiple questions in human biology and medicine that could be insufficiently addressed using conventional biofabrication techniques[24]. Recent advances in bioprinting techniques, material science, cell biology, and many other disciplines have increased the possibility for engineering novel *in vitro* model system to reflect organ physiology and disease states as closely as possible. In the last few decades, significant progress has been made in the bioprinting of *in vitro* vascularized tissue models for applications to biomedical research and drug screening. Despite the significant advances described above, the structural, cellular, and molecular features of vascular networks have yet to be fully achieved. With current bioprinting approaches, the clinical availability of bioprinted *in vitro* models is still elusive, and important practical challenges need to be addressed, including the construction of fully functional multi-scale vasculature, the assembly of diverse cellular populations and tissue-specific matrices, and prolonged functionality.

In general, extrusion-based approaches have not yet led to the fabrication of refined, fully functional multi-scale vasculature due to the lack of resolution and precision in the printed tissue constructs. Most vital organs, including the liver, kidneys, lungs, and brain, have a rich vascular system representing a complex hierarchy of dimensions and compositions, from micro-scaled capillaries to millimeter-sized vessels. Thus, more advanced bioprinting strategies with improved resolution and precision are necessary to produce complex and scalable human vasculature. In addition, replicating the cellular and compositional heterogeneity of vascularized tissues in an organ-specific manner is critical. Most of the reported bioprinting model studies have been demonstrated by incorporating tissue-relevant parenchymal cell lines, thus making it difficult to fulfill the organ-specific requirements. The advancement of stem cell technology has allowed the isolation of induced-pluripotent stem cells and organoids from adult or fetal tissue biopsy samples[70]. Leveraging high-quality human cells, such as tissue-resident stem (embryonic or adult) or induced-pluripotent stem cells and organoids, will pave the way for a paradigm shift in developing more reliable *in vitro* models that emulate critical aspects of organ-specific physiology and function. Moreover, the selection of ink materials that provide cells with a microenvironmental niche is essential for effective cell growth and function[22,71,72]. Among prevailing ink materials used in 3D bioprinting, tissue-specific dECMs have received increased attention as the most promising biomimetic ink material that can better provide the intrinsic natural

properties of their native counterparts[72-74]. In this context, incorporating tissue-specific dECMs could lead to the bioprinting of complex and biomimetic models with more improved biofunctionality. The success of bioprinted models will largely rely on their level of maturity and sustainable functionality. Future bioprinted vascularized models should accommodate all biomimetic aspects, including pre-defined multi-scale vascular organization, heterocellular compositions, and coordinated stimulating factors toward organ-level complexity.

In summary, 3D bioprinting has opened up a promising route to model human biology and diseases *in vitro*. As a next-generation research platform, 3D-bioprinted *in vitro* models are now poised to make a significant impact on the clinic to accelerate drug development and serve as living avatars for personalized medicine.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

Conceptualization: Suhun Chae, Hyungseok Lee
Funding acquisition: Dong-Heon Ha, Hyungseok Lee
Supervision: Hyungseok Lee
Visualization: Suhun Chae
Writing – original draft: Suhun Chae
Writing – review & editing: Dong-Heon Ha, Hyungseok Lee

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