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Mimicking the liver function in micro-patterned units: Challenges and perspectives in 3D bioprinting

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ABSTRACT

Bioprinting of 3-dimensional (3D) micro-patterned structures could be a promising approach to mimic complex organs *in vivo*. This technique holds promise for generating micro- and macroscale bioengineered structures in order to produce a platform which will be closest to the original tissues. However, there are many challenges regarding liver tissue engineering, due to the high vascularization of the liver tissue, problems with culturing hepatocytes, and the complex microenvironment of the liver. Here, the current challenges in the bioprinting technique for the liver tissue and their potential solutions are discussed. The most suitable cell types, different bioprinters, and various hydrogels are described.

1. Introduction

The liver as the largest internal organ, which plays a vital role in different physiological and metabolic functions. Accordingly, any disorder affecting the liver functions leads to a wide range of pathologies [1]. Partial or the whole organ transplantation has arisen as a gold standard treatment for patients with liver failure. However, liver transplantation faces some limitations such as the shortage of donor organs and insufficient engraftment success [2]. Other available solutions such as bioartificial liver systems or cell therapy approaches can be used as only a temporary support with low therapeutic results [3,4]. In this regard, liver tissue engineering has offered an alternative solution based on the 3D bioprinting technique, which involves creation of cell laden- and scaffold-based constructs with complex geometries to manufacture functional units [5]. Merging this technique with printable biomaterials, appropriate cells and growth factors allows one to create a more developed model for a better understanding of the biological complexities at the cellular and molecular levels [6]. Considering the recent improvements in 3D bioprinting protocols, it was shown that bioprinted liver tissue could overcome the current limitations in liver transplantation, liver regeneration and high throughput drug assays [7–9]. However, the construction of complex 3D tissues like the liver has always been a driving force [10].

Over the past few decades, the field of liver bioprinting and tissue engineering has been significantly developed with a better understanding of the cellular phenomenon, liver microstructure and microenvironment, stem cell differentiation technologies, various aspects of material science and fabrication and design methodologies [2]. For example, while creation of larger 3D constructs can better restore liver functions, the size of the engineered tissue is limited to 100–200 μm due to diffusion limits of O_2 and nutrients [11]. To overcome such limitations, sacrificial materials have been introduced in tissue engineering as a temporary factor, in order to generate microchannels [12–14] or coaxial nozzles for the formation of blood vessels [15,16].

In this perspective, we highlight the current challenges and limitations of the 3D bioprinting technique in regard to bioprinters, different cell sources, various matrices, and vascularization-related problems.

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2. Hydrogels as bioinks and their challenges

In the several past years, many natural and synthetic polymers have been introduced for the use as a hydrogel base of a bioink (Fig. 1). Among them, natural polymers are the desired choice for bioprinting and engineering soft tissues including the liver, which has a stiffness of approximately 1.5–2 kPa [17]. In a number of studies, different hydrogels have been chosen alone or in combination to improve the hepatocyte functionality, enhance the differentiation rate, generate disease modeling, and for other applications related to the liver regeneration.

Here, we discuss the important criteria that should be considered in bioprinting to choose a proper hydrogel. An ideal material for a bioink should [1]: be biocompatible without any toxicity and not induce immunological reactions [7] have the corresponding mechanical stability of the organ that should be printed [18] have a capacity of adhering to cells [9] have acceptable biodegradability [10] recapitulate the natural environment related to specifically used cells [19] have the ability to support the key cellular activities such as migration, proliferation, differentiation and adhesion [20,21].

Biodegradability, as well as the stiffness, are crucial for all types of biomaterials related to individual tissues [22,23]. Practically, biodegradability is associated with the re-establishment of the tissue functions, while the stiffness is related to numerous cellular activities such as differentiation, proliferation, and tissue regeneration [24,25]. Another important property of a hydrogel is biocompatibility. The hydrogel must be non-toxic when printed along with cells. Moreover, cytoadherent polymers are required to provide the structural support for cell proliferation or expansion [26–28]. Another concern for such polymers is their printability, which is related to the viscoelastic or rheological properties of hydrogels. Different printing methods need different viscosity in individual hydrogels. Viscosity acts as a double-edged sword; high viscosity results in more stable printed structures, but increases the flow resistance of the bioink from the printing nozzle. In this regard, more pressure is needed to inject the bioink, which may result in clogging the nozzle and impairing the cell membrane [27,29]. In this section, we discuss the properties of the most commonly used synthetic and natural polymers used as bioinks.

Synthetic polymers including polyethylene glycol (PEG), poly lactic-co-glycolic acid (PLGA) and polycaprolactone (PCL), which are more common in liner bioprinting, are non-cytoadherent. To overcome this drawback, their surfaces can be modified to provide cell adhesion [26]. Among them, PEG hydrogels reveal a high water preservation capacity, which is similar to that of the liver, and excellent solubility in water, while PCL and PLGA are not adequately soluble in water; however, they have very flexible mechanical properties that makes them a good choice for engineering hard and soft tissues.

PEG is an FDA-approved biocompatible polymer with non-immunogenic properties. However, low viscosity of a PEG solution makes it inferior for the use in extrusion-based and inkjet printers [17]. PEG hydrogels are widely used as a biocompatible matrix for encapsulation of primary hepatocytes to increase their survival rate [30]. To improve the survival rate and functionality of hepatic cells encapsulated within a PEG hydrogel, some modifications of the polymer chain and conjugation of certain bioactive factors were applied with promising results [31]. To improve the hepatic differentiation, PEG was used in 3D hexagonally arrayed lobular liver tissues. The organization of a hydrogel in this format facilitated self-assembly of primary human fetal liver cells into a 3D structure and conserved the hepatic functions for at least 5 months [32].

PCL is a nontoxic, biocompatible, very stiff (not suitable for the liver) polymer with slow biodegradation. Moreover, the insoluble nature of PCL limits its usage for immediate printing of cell-laden structures [33]. Interestingly, PCL has been used in a combination with a variety of natural polymers [34]. For example, Rhiannon et al. created hybrid PCL/extra cellular matrix (ECM) scaffolds, which could preserve hepatocytes' function and growth [35]. Moreover, PCL has been used for bio-printing of hepatocytes with some stromal cells such as human umbilical vein endothelial cells (HUVECs) and human lung fibroblasts. This co-culture resulted in maintained hepatocytes' functions and facilitated formation of the vascular networks [36].

PLGA is also an FDA-approved polymer that can be manipulated to adjust the degradation time. Besides, rapid hydrolysis of its ester bonds in water can restrict the use of water as a solvent for PLGA, which limits printing of cell-laden constructs [37]. However, due to the biocompatibility and controlled biodegradability of PLGA, its microspheres have been applied as scaffolds comprising cells to improve vascularization in engineered constructs [38]. In one of studies, a 3D stacked culture method was developed using biodegradable PLGA membranes with defined topography. When hepatocytes were cultured with PLGA, the cells restored their polarity and showed enhanced liver-specific functions, as well as exhibited highly differentiated functions as compared to cells cultured in a monolayer [39]. Moreover, more recently, a PLGA polymer has been used to construct an absorbable vascular anastomosis device. The device was tested in pig liver transplantation tests, and the data showed that it was successfully absorbed in 4 months, while the anastomosis remained functional [40].

The most commonly used natural hydrogels for liver tissue bioprinting are alginate, collagen, gelatin, and decellularized ECM (dECM). Alginate is extracted from brown algae and, due to its non-immunogenic properties, can be a reliable hydrogel for liver bioprinting [41]. Alginate

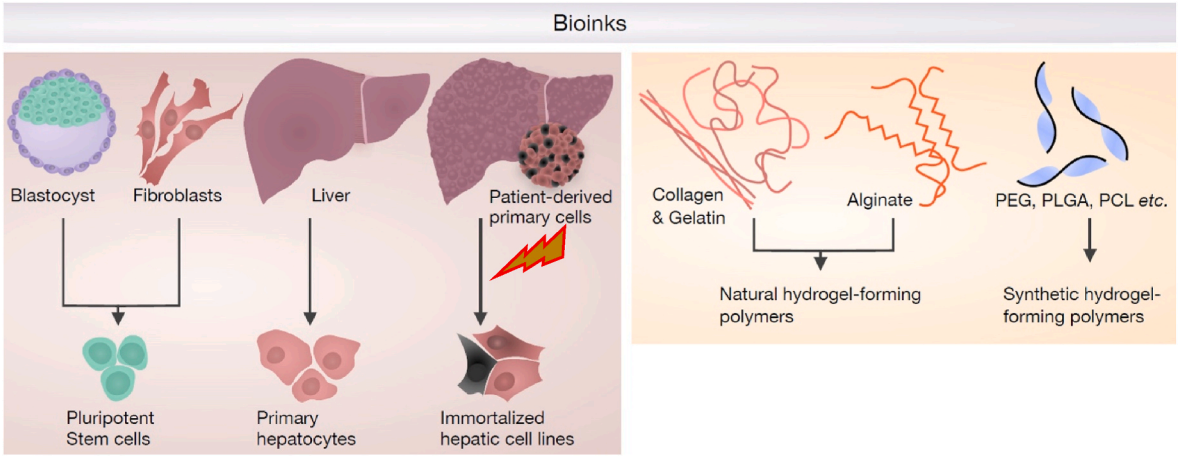

Fig. 1. Schematic representation of different cell sources and common natural and synthetic hydrogels in liver bioprinting. Pluripotent stem cells can be derived from both fibroblasts (reprogramming) and blastocysts (inner cell mass). Primary hepatocytes and hepatic cell lines are isolated from healthy and cancerous livers.

is a water-soluble hydrogel, however, the sol–gel transition occurs at a temperature below 0∘C, that restricts physical gelation after printing at the physiological or room temperature [33]. Alginate has an excellent formability, adequate biocompatibility and is able to encapsulate active biomolecules for delivery [41]. One of the major challenges in the creation of satisfying in vitro models is the lack of vascular networks. Christensen et al. fabricated vascular-like cellular constructs using a liquid support-based inkjet printing method, which employed a calcium chloride solution as both a cross-linking agent and a supporting material. Vascular-like structures were successfully printed and formed vertical and horizontal bifurcated features from sodium alginate alone and from mouse fibroblast-containing alginate bioinks. The cell viability of fibroblasts in printed cellular tubes was 92.4% immediately after printing and approximately 90% after 24 h of incubation [42]. Moreover, a combination of alginate with other polymers such as gelatin methacryloyl (GelMA) has been used for vascular tissue engineering. A cell-responsive bioink was designed containing GelMA, sodium alginate, and 4-arm poly (ethylene glycol)-tetra-acrylate (PEGTA) for the use with a multilayered coaxial extrusion system. To form stable structures, this composite bioink should be ionically crosslinked using calcium ions, then further stabilized by covalent photo-crosslinking. The designed bioink presented many biological and mechanical advantages. These properties supported spreading and proliferation of encapsulated HUVECs and stem cells in the bioprinted structures [43]. Apart from its favorable properties, alginate is non-cytoadherent and its stability in post-printed structures mainly depends on the degree of crosslinking by the divalent cation. However, alginate-based structures are very sensitive to pH that can disturb the ionic interaction between the polymer and the ion. Finally, the functionality of structures bioprinted using alginate depends on the polymer concentration, degree of crosslinking, and cell density [44–46].

Another ideal natural polymer for encapsulating hepatic cells is collagen, which is the main component of the ECM, and its 19 subtypes are responsible for various physiological functions [47]. Although collagen is a highly biocompatible and cytoadherent polymer, its mechanical strength is rather poor that results in a collapse of the created scaffolds [26,28]. Collagen supports proliferation, cell adhesion, migration and differentiation due to the arginine-glycine-aspartic acid (RGD) motif [48]. Lee et al. introduced printing of a 3D cell-laden construct using the 3D cell printing technology and a multi-head tissue building system. They used PCL as a framework material due to its proper mechanical properties. Rat primary hepatocytes, HUVECs, and human lung fibroblasts incorporated into collagen were utilized as a bioink infused into the PCL canals to induce the formation of capillary-like networks and to promote the liver cell growth. Interestingly, a vascular system was formed and hepatocytes' functions such as albumin secretion and urea synthesis were reported [49].

On the other hand, hydrogels containing only collagen are not appropriate for 3D bioprinting because of their uncontrolled gelation characteristics, poor mechanical properties, and limited printability. To address such obstacles, some studies included development of various collagen-based bioinks [50] or specific cross-linkers for liver bioprinting [48]. For example, collagen type I is the main structural component in the ECM and has been commonly used as a hydrogel, however, due to its low viscosity and slow polymerization, it is less appropriate for 3D bioprinting applications. In a study by Mazzocchi et al., a collagen type I/hyaluronic acid hybrid hydrogel bioink was created, which was employed to bioprint 3D liver tissues including primary human hepatocytes (PHH) and liver stellate cells. The obtained results showed that this formulated printable bioink enabled elongation of stromal cells and acted as a supporting hydrogel for incorporation of hepatocytes, which were maintained viable for over two weeks and responded to the drug treatment [51]. Collagen gelation at a physiological temperature (~37 °C) takes time (about 30 min) which makes its broad application limited [52]. Furthermore, another obstacle that limits the use of collagen in bioprinting is its high biodegradability, especially after transplantation [53].

Another interesting bioink in liver bioprinting is gelatin, which is derived from collagen by partial hydrolysis. The properties of gelatin, including biocompatibility, excellent solubility in water, printability, biodegradability, low immunogenicity, and the RGD motif, which is important for cell attachment (can support certain cell activities such as cell adhesion, migration, and proliferation) make it an excellent candidate for biomedical applications [54,55]. Moreover, the sol–gel transition temperature of a gelatin solution is approximately 28∘C (25–30∘C) that has made it a reliable natural bioink for 3D organ bioprinting [56]. GelMA is a photosensitive gelatin with excellent formability and biocompatibility [57]. GelMA scaffolds have the desired stiffness and high shape reliability when used in liver bioprinting [58]. Ma et al. applied GelMA with a stiffness comparable to that of a healthy liver to support the cellular functions and behaviors, such as migration and proliferation [59]. Furthermore, a combination of glycidyl methacrylate hyaluronic acid (GMHA) and GelMA was used to provide a more bioactive microenvironment for endothelial cells, in which hyaluronic acid promoted proliferation of endothelial cells and facilitated vascularization [60]. More recently, a drop-on-demand bioprinting approach to produce HepG2 cell suspension droplets on a polyethylene terephthalate substrate has been optimized. The substrates carrying droplets were placed inside a novel Hanging-drop Culture Chamber (HdCC) and incubated to obtain the liver parenchymal microtissues with a size of 200–300 μm. The microtissues were viable, exhibited an active response to insulin stimulation, and expressed polarity markers including multidrug resistance protein 2 (MRP2) and zonula occludens 1 (ZO1). In addition, to preserve the structural integrity and improve their functional abilities, the microtissues were sandwiched in the GelMA hydrogel, and the liver-specific functions were assessed during 2 weeks. The results showed that the 3D structure of the microtissues in the GelMA sandwich conserved the albumin secretion, urea synthesis, and cytochrome P450 activity, compared to microtissues in a suspension [61]. In another study, a GelMA hydrogel was applied as a matrix to construct 3D lobule-like microtissues containing hepatocytes and fibroblasts. The experimental results confirmed that 3D lobule-like microtissues constructed using GelMA hydrogels showed high cell viability and liver function, that revealed a promising potential for liver tissue bioengineering and regenerative medicine applications [62]. Nevertheless, GelMA-based bioinks have not been yet approved for clinical trials due to their cytotoxicity.

Another promising natural hydrogel is dECM that has recently been considered as a proper biomaterial for bioprinting applications. The tissue-specific ECM plays an essential role in cellular proliferation, anchorage, migration, and signaling [63]. The natural liver ECM obtained after a chemical and/or enzymatic process contains a wide range of proteins, collagen, proteoglycans, and glycosaminoglycans with bioactive molecules. This complex can mimic the *in vivo* microenvironment and support the cellular functions compared to single protein hydrogels [64,65]. Gelation in dECM usually occurs at a physiological pH and temperature, which makes it an appropriate candidate for the use in 3D bioprinting. However, low viscosity, poor shape retention, and rapid biodegradation of the dECM hydrogel limit its application in bioprinting of large solid organs. Besides, the common dECM source is xenogenic, in particular, porcine, which may pose immunogenicity *in vivo* [64]. Nevertheless, application of dECM would be the ideal scenario for printed cells to recapitulate the native hepatic structures [66]. A technique capable of fabricating the liver and heart microarchitecture with high control and appropriate mechanical properties has been developed, which uses photocrosslinkable tissue-specific dECM as a bioink (heart and liver), dynamic light processing (DLP) as a bioprinter and human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes and hepatocytes as cell sources. The dECM bioinks provided a conducive microenvironment to improve maturation and maintain the viability of hiPSC-derived cardiomyocytes and hepatocytes. This micropatterning also directed the cellular self-organization

into the pre-designed striated heart and lobular liver assemblies with biophysical cues [67]. Due to the low mechanical stability and slow gelation mechanism of dECM, the construction of complex structures is challenging [68,69]. Therefore, approaches to enhance dECM bioinks' printability are studied, for example, a combination with other cross-linkable hydrogels [70,71], adding photo-crosslinkers [72,73], and introduction of the methacrylate group [74]. In this regard, Ma et al. used a combination of the liver dECM and GelMA to improve a photo-crosslinkable dECM-based bioink for DLP-based 3D bioprinting. As a result, the dECM crosslinked *via* the GelMa photocrosslinking demonstrated high fidelity, and the printed shape remained stable for 7 days. The printed scaffolds were able to mimic the mechanical properties of the cirrhotic liver tissue. The growth rate of HepG2 cells encapsulated in dECM/GelMA scaffolds with the cirrhotic-like stiffness was reduced, while the expression of the invasion markers was increased, compared to healthy controls [70]. In another study, Kim et al. adjusted the optimal ratio of the liver dECM and a gelatin-based mixture (gelatin, hyaluronic acid, and fibrinogen) for extrusion-based 3D bioprinting. The results showed that the mechanical properties and 3D printability of this multicomponent bioink were greatly improved and the liver-specific markers were expressed similar to those in the cells encapsulated in the liver dECM [75].

2.1. Bioprinters and their challenges

Depending on the particular goal – fabrication of a full-size organ or of the liver-on-a-chip – different types of bioprinters or their combinations may be applied. At any rate, while bioprinting the liver, first, its morphology should be recapitulated, which represents a complex hexagonal shape with periportal, midzonal, and perivenous areas and highly developed vasculature (Fig. 2).

To construct the main tissue structure, extrusion bioprinting is commonly applied, and various bioprinters are commercially available (e.g. BioBot, BioX, etc.). Such bioprinters ensure high viability of hepatocyte-like cells, which can reach 98% in 2 weeks after bioprinting [76,77]. Both single cell suspension or cell spheroids can be used as a cell component of a bioink [78]. Compared to other types of bioprinting, extrusion bioprinting enables fabrication of highly rigid and stable vertically oriented structures. For instance, hepatorganoids with the sizes of $10 \times 10 \times 3$ mm were successfully formed and shown to increase the survival rate in mice with liver failure [79]. Moreover, new set-ups have enabled a higher resolution (up to 1 μm [76]) and the simultaneous use of several types of bioinks [78], that provides an opportunity to fabricate more complex structures. Particularly, the hepatic lobule-like structure was shown to be recapitulated using a pre-set extrusion bioprinting equipment with precursor cartridges containing different materials [80]. Nevertheless, the main disadvantages of extrusion-based bioprinting in respect to creating liver-like constructs are a relatively low printing speed, nozzle clogging, a suboptimal processing temperature, and ultraviolet (UV) exposure required to cross-link a bioink. These factors may significantly decrease the cell survival rate (10%) and cause structural defects [77,79,81].

Inkjet bioprinting is another commonly used option which enables higher resolution and speed and, therefore, higher cell survivability than extrusion bioprinting does [20]. This method was successfully applied to fabricate liver-like constructs for high-throughput drug screening [82]. However, inkjet bioprinting usually works with a relatively low cell density which does not exceed 10^7 cells/mL [83,84].

Other techniques such as laser-assisted or photocuring-based bioprinting can be recommended to recapitulate the particular structures in the liver tissue (including the bile duct, the space of Disse, and the vasculature) in more detail and can be efficiently coupled with microfluidics [85]. Compared to those mentioned above, these types of bioprinters provide the highest resolution [86]. Laser-assisted bioprinting can apply bioinks with the highest cell density (up to 1×10^8–1×10^9 cells/mL [87]) or spheroids [88], that ensures the formation of well-organized liver-like units [89]. Nevertheless, this method has low scalability, and the fabrication of the whole organ can be hardly achieved. Photocuring-based bioprinters are presented by stereolithography (STL), DLP, and holographic printers [90] and were shown to allow the formation of finely defined structures like the vasculature [91] and hexagonal lobule-like blocks with a thickness of 200 μm [18]. However, photocuring-based bioprinting has many disadvantages among which is the exposure to UV light and potential toxicity of photocuring agents (light absorbers and photoinitiators) [92,93]. These factors cause relatively low post-printing cell survivability, which usually does not exceed 85% [91,94,95]. Moreover, the high cell density can decrease the photopolymerization efficiency and cause defects in the formed structure [91].

To increase the similarity between the original and printed liver, other techniques were also developed, e.g. coaxial bioprinting [96], chaotic flow printing [97], 4D printing [98], etc. Coaxial bioprinting was reported to facilitate mimicking the anisotropic morphology by enabling the in vivo-like cell growth [96]. Acoustophoretic bioprinting can ensure the formation of a complex structure and help to avoid the shear, thermal, and UV stress [99]. Chaotic flow printing enables achieving a high printing speed with a resolution of 10 μm at an area of up to 10 cm^2 [100]. It was shown to be an efficient tool to fabricate a 3D microvascular network [101]. Moreover, complex liver structural patterns can be formed via 4D printing using smart (stimuli-responsive) materials, and the possibility to produce 3D hexagonal-like constructs has already been reported [101–104]. (Table 1).

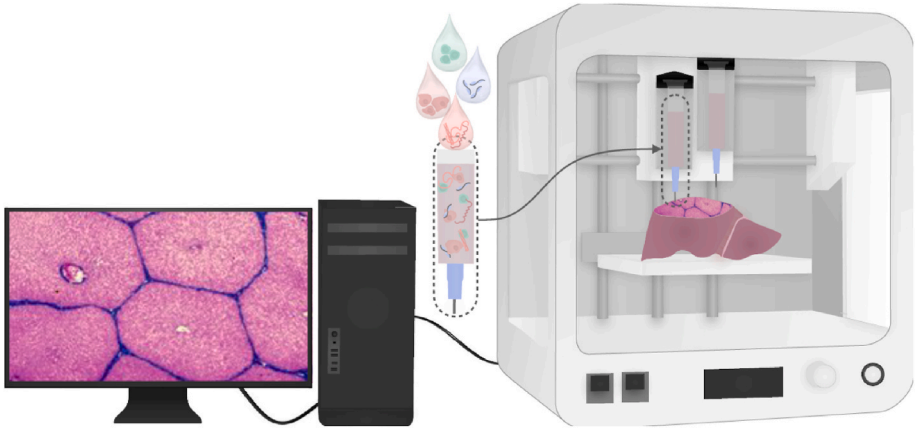

Fig. 2. An overview of the 3D bioprinting process. Stages (from the left to the right): computer-assisted designing, pre-printing (including cell selection, hydrogel synthesis, and bioink blending), bioprinting.

Table 1
Characteristics of different bioprinting set-ups.

Examples	Resolution	Sizes	Printing speed	Cell viability	Cell density	Drawbacks	Ref.
Extrusion bioprinting							
Allevi extrusion printer	100–200 μm	7 × 7 × 2 mm	–	~100% (4h AP)	Medium	• UV light exposure •Pressure	[81]
Pneumatic extrusion printer	–	10 × 10 × 1 mm	–	–	7×10^6 cells/ml	•Low temperature •Nozzle clogging	[105]
SPP1603	–	10 × 10 × 3 mm	150 mm^3/min	up to 90% (IM AP)	1×10^6 cells/ml	•Hypoxic areas in spheroids' cores	[106, 107]
BIOX	1 μm	3 × 3 × 2 mm	–	99% (1 d AP) 98% (14 d AP)	3×10^7 cells/ml		[108]
INKREDIBLE+	100 μm	–	–	80% (18 d AP)	1×10^7 cells/ml		[109]
Inkjet bioprinting							
Custom-made inkjet printer	80 μm	9 × 9 × 5 mm	30 mm/s	–	Medium	•Low mechanical properties •Shear stress	[110, 111]
Piezo-electronic inkjet printer	25 μm	1 mm (⌀)	–	~100%	up to 1×10^7 cells/ml	•Thermal stress •Clogging	[112]
Lab-on-a-printer inkjet dispenser	150 μm	18 × 26 mm	1.2–1.7 mm/s	–	Medium		[113]
Laser-assisted bioprinting							
LIFT experimental setup	25–100 μm	–	High	~100%	High	•Only low and medium viscous bioinks can be applied	[114, 115]
LIFT experimental setup	50 μm	–	200 mm/s	High	1×10^8 cells/ml	•Possible contamination with metal particles	[116]
LIFT experimental setup	High	200–300 μm (⌀)	High	~80% (3 h AP; iPSC)	1×10^9 cells/ml	•Difficult process scalability	[117]
Photocuring-based bioprinting							
Custom-made projection stereolithography set-up	5 μm	530 × 740 × 360 μm (1 block)	–	70% (1 d AP)	up to 1×10^7 cells/ml	•UV light exposure •Difficult to print more than 1 material •Toxic photocuring agents	[118]
High-resolution stereolithography set-up	50 μm	1.5 × 1.5 × 2.4 cm	0.56 mm^2/min	85% (5 d AP)	Medium		[119]
Perfactory® 3 Mini	30 μm	5 × 5 × 5 mm	5 μm/s	70% (21 d AP)	5×10^6 cell/ml		[120]
Custom-made digital micromirror device (DMD)	–	3 × 3 × 0.2 mm	–	~70% (1 d AP) ~60% (7 d AP)	4×10^7 cells/ml		[121]

Abbreviations. AP – after printing, IM – immediately, LIFT– laser-induced forward transfer, iPSC – induced pluripotent stem cells.

Thus, to recapitulate the liver morphology as close as possible, the use of one printing technique cannot be sufficient. From our point of view, bioprinting of the liver requires designing a special platform which does not harm liver tissue cells sensitive to any stress and ensures rapid fabrication of the main tissue via extrusion-based bioprinting with the precise deposition of minor cell populations in accordance with the complex tissue-specific patterns by laser-assisted, photocuring-based, or 4D printing.

3. Cell sources and their challenges

The liver is composed of parenchymal cells, namely, hepatocytes and cholangiocytes, and non-parenchymal cells, i.e., hepatic stellate cells, Kupffer cells, and endothelial cells. Hepatocytes are associated with the major functions of the liver, and non-parenchymal cells support the liver parenchymal cells [122]. In this section, we discuss the potential sources of hepatocytes used in liver bioprinting and their challenges.

Despite the recent advances in the cell culture protocols, employing the best hepatic cell type for bioprinting is still a major challenge due to the limitations of in vitro expansion, sustaining primary hepatocytes' functionality for long periods of time, the insufficient differentiation rate in hepatic-like cells (HLCs) and so on [123,124]. Primary hepatocytes (human or animal) isolated from a healthy liver, are known as a gold standard cell source for hepatic bioprinting because of their high metabolic activities [125]. Due to the increasing number of patients who suffer from end-stage liver disease and the lack of donors for liver transplantation, many studies focus on alternative treatments, such as primary hepatocyte implantation. In this case, the required number of primary hepatocytes can be obtained from a single donor, that enables the treatment of several patients. Moreover, isolated cells can be cryopreserved and stored for future applications [126,127]. To predict the hepatotoxicity of new medicinal components in the liver tissue, PHH are a gold standard owing to their ability to preserve their metabolic profile [65]. However, due to the limited access to primary hepatocytes and their rapid dedifferentiation, cultivation of primary hepatocytes is still challenging. This happens because of the deprivation of primary hepatocytes of their natural microenvironment with a cell-cell contact with stromal cells [128]. To reproduce this natural environment, the recent studies have reported co-culturing primary hepatocytes with supportive cells, and also different matrices in a 3D culture [65,124,129]. To achieve and recover the native microenvironment, liver tissue engineering, especially 3D bioprinting, has been developed in the recent years (Table 2, Fig. 3). In one of the studies, a 3D construct was fabricated using 3D cell printing technology, a collagen solution filled with hepatocytes, human lung fibroblasts and HUVECs being used as a bioink and PCL as a framework. The authors confirmed the concept that a co-culture of hepatocytes with non-parenchymal cells was essential for their maintenance and for increasing their survival rate compared to a single culture. A 3D cell-printed construct comprising a network of capillaries improved the protein secretion and metabolism of primary hepatocytes [49]. In the field of cell-based therapy and transplantation using primary hepatocytes, another study demonstrated that the 3D bioprinting method combined with a microneedle array system for fabricating a liver-like tissue, composed of primary hepatocytes, mesenchymal stem cells (MSCs) and HUVECs, could generate hundreds of liver bud-like spheroids [130]. Some drug toxicity studies used patient-derived primary hepatocytes combined with 3D bioprinting as a model to demonstrate the drug response [78]. Furthermore, in some studies the bioprinting technology was applied for creating a liver model composed of primary hepatocytes, human hepatic stellate cells, and other non-parenchymal cells, to model a liver injury leading to fibrosis [131, 132].

In summary, although human hepatocytes represent a gold standard for studies of hepatotoxicity and transplantation, hepatocytes' culture

Table 2
Summary of studies using various types of bioprinters and bioinks for liver tissue models.

Bioprinting strategy	Bioink		Outcomes	Ref.
	Cells	Hydrogel materials		
Extrusion	Primary human hepatocytes/Liver stellate cells(LX2)/aHSC	Methacrylate collagen type I/Thiolated hyaluronic acid	The bioink acted as a support hydrogel for hepatocytes and the cells could stay viable and secret albumin and urea for more than 2 weeks, as well as responded to drug intervention.	[50]
Extrusion	PMHs	Porcine liver-derived dECM	The effect of different decellularization methods on the functionality of 3D bioprinted liver construct were investigated.	[158]
Extrusion	HepG2, HUVEC, MSCs and HFF cells	Gelatin (sacrificial material)/GelMA –Fibrin (Cell- laden)	The liver's nutrients and oxygen were efficiently fulfilled by 3D-printed capillary structures.	[159]
Extrusion	HepG2	GelXA lamininK521 (gelatin, alginate, xanthan gum and laminin a5b2y1)	The hexagonal liver microstructure bioprinted for developing in vitro models with the long-term cell growth.	
Extrusion	HepG2-laden in shell bioink/NIH 3T3-laden in core bioink	algMC + Matrigel/algMC, algMC + fibrin or algMC + plasma	The liver core–shell bioprinting model containing hepatocytes and fibroblasts was established that showed the production of hepatocyte markers such as albumin.	[160]
Extrusion	Primary HCC cells	Gelatin/Alginate	A 3D-HCC model with a patient's cells showed the long-term survival and maintenance of a patient-specific genetic profile.	[161]
DLP	hiHep cells	GelMA/porcine liver dECM	The constructed model can be utilized in the regeneration of the liver and restoration of liver functions.	[68]
Extrusion	HepaRG cells	Gelatin and Alginate	In animal models, this functional liver microstructure-tissue demonstrated the normal liver functioning for up to 7 days after transplantation. In addition, the combination of the ink and printed cells may enhance the vascular structure in the transplanted structure.	[106]
Extrusion	HepG2/NIH/3T3	GelMA/135ACG hybrid (Alginate-Cellulose nanocrystal-GelMA)	Printing of hepG2 and NIH/3T3 cells in the 135ACG and GelMA matrix showed advancement of fibroblast cells in the 135ACG matrix, whereas hepG2 produced exclusively spheroids with high albumin secretion, higher functionality and interaction with other cells.	[162]
Microfluidic- based bioprinting	HepG2/3T3-J2	PEG	Direct 3D printing of HepG2 and 3T3-J2 cells onto an established fibroblast layer in the novel microfluidic method led to their gene expression and albumin secretion being nearly twice as high as that in the 2D conditions.	[163]
Extrusion	HepG2/C3A + ECs	Collagen/Alginate	It was revealed that each cell type in the bioink with spatial cell patterning accelerated cellular organization, which may preserve the structural integrity while increasing the cellular activity.	[164]
Inkjet	NIH3T3/HUVEC	Porcine Liver dECM /Gelatin	The cells in this construct were viable, surviving in the printed ink at a rate of 93%.	[75]
Extrusion	ICC	Gelatin/alginate/Matrigel ™	The expression of cancer markers in patient-derived cells, functional markers of liver cells, and proteins associated with metastasis and invasion were important features of this structure. Furthermore, the response of these cells to drug therapy and drug resistance was comparable to that *in vivo*.	[165]
Stereolithography (	HepG2/NIH/3T3 fibroblast	GelMA	3D microtissues provided long-term co-culture of hepatocytes and fibroblasts with excellent cell viability and an improved albumin secretion function in vitro.	[62]
Extrusion	iPS- derived hepatocyte-like cells spheroids/MSC/ ECs	Alginate/Pluronic F127 blend bioink	18-day-long 3D printing of iPS-derived hepatocyte cells in combination with other cells such as mesenchymal stem cells and endothelial cells (spheroids) as support cells improved the cell survival, metabolic activity stability, and hepatocyte phenotypic preservation	[109]
Stereolithography	hiPSC-derived hepatocytes	Liver dECM	Microstructure patterning facilitated spontaneous cellular rearrangement into the predesigned lobular liver structures via biophysical signals.	[166]
Extrusion	Primary human hepatocytes/HSCs/ECs/ KCs	NovoGel 2.0 Hydrogel	TGF-b1 and methotrexate (MTX) therapy for 28 days resulted in the identification of hepatic injury and increasing fibrosis as evidenced by the production of fibrillary collagens in patterns, reduced albumin synthesis, which was consistent with the previously observed LDH and miR-122 responses.	[167]
Extrusion	HepG2/C3A	Pluronic F127/Alginate hydrogel	Following the acetaminophen treatment, the 3D printed structure demonstrated improved hepatic functions and sensitivity, indicating more physiologically key features for in vitro drug research using 3D hepatic systems.	[168]
DLP	HepG2 cells	Liver dECM	Liver photocrosslinkable dECM utilizing the DLP method manifested itself as an appropriate platform in the form of liver lobule architectures to explore the influence of cirrhosis and healthy liver matrices on the cell behavior such as invasion and survival.	[169]
Extrusion	HepaRG	Alginate/Gelatin/hLdECM	Cyclophilin silencing as a target gene of adenovirus 5 verified the effectiveness of the manufactured model in transduction and future antiviral drug testing.	[170]
(SLA) laser-based direct-writing	HepG2	Gelatin-(transglutaminase)TG hydrogel(cell laden hydrogel)/PLA(support structure)+ PVA(Sacrificial templet)	A 3D structure with four arm branch network was designed for growing vascular tumor model.	[171]
Extrusion	Mice primary hepatocytes/UCB-MSC	Alginate		[172]

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Table 2 (*continued*)

Bioprinting strategy	Bioink		Outcomes	Ref.
	Cells	Hydrogel materials		
			Both the 3D bio-printing method and paracrine chemicals produced by MSCs allowed for the long-term hepatocyte viability for 1 week without morphological alterations.	
Stereolithography (DLP)	HepaRG/Human HSCs	GelMA/PEG	The 3D-printed liver exhibited enhanced metabolic activity as compared to a monolayer HepaRG culture. The overall metabolism, as well as the liver-specific bile transporter and MRP2, were shown to be stable.	[173]
Extrusion	Huh7	Gelatin	In terms of the albumin secretion, cytochrome p450 oxidase activity, and bile salt transport activity, the 3D structure with a 60° geometry is preferable and could improve these activities when compared to a 2D model.	[174]
Inkjet	PMHs	GA	A GA-gel biocompatible hydrogel that interacts with hepatocytes via the ASGPR can be used as an advantageous 3D culture system for engineering of the liver tissues, maintaining the cellular polarity and adhesion and studying cellular responses.	[110]
Extrusion	HepG2	Alginate	HepG2 cells labeled with the mCherry red fluorescent protein to efficiently track their location and detect their proliferative ability were significantly positive for the hepatic indicators including albumin and AFP production. After 21 days, a 3D-printed scaffold allows HepG2 cells to repopulate without losing their hepatic identity.	[175]
Extrusion	BMMSCs/HepG2 cells	Porcine liver dECM, PCL, collagen	The liver dECM bioink for 3D-hepatocyte printing was shown to create a suitable microenvironment with the desired biochemical and biomechanical properties, that significantly improved stem cell differentiation and liver cell functions over the available collagen bioink.	[176]
Extrusion	miHeps	Alginate	MiHeps were printed using an alginate bioink, grown in vitro for a week, and then implanted *in vivo* in a mouse with a liver injury model. The gene expression data for albumin, ASGR1 and HNF4a and the immunofluorescence assay after 4 weeks showed the restored liver functions.	[177]
Extrusion	HepG2/C3A/HUVECs	GelMA	The presence of a HUVEC layer causes the increased viability and metabolic activity of HepG2/C3A cells within a 3D-printed liver.	[178]
Extrusion	Human hepatoma cells/ Fibroblasts	Alginate/CNCs	The fabrication of a 3D liver-mimetic honeycomb construct incorporating alginate and CNC as a hybrid ink demonstrated good shear thinning and printable properties, as well as low hepatoma and fibroblast cell mortality.	[179]
Extrusion	hASCs/derived AHLCs	Collagen	Cell translocation from the construct to rats' hepatic portal veins observed after biofabrication of hepatic blocks in 3D printed structures containing hASCs, AHLCs, and collagen, followed by implantation in SD rats after 4 weeks. The analysis of liver-specific parameters revealed that this 3D liver model aids in the treatment of rat liver failure.	[180]
Extrusion (organ-on-a-chip)	HepG2/HUVEC	PCL microfluidic channel/Collagen/gelatin	The inclusion of a variety of cell types in this proposed approach, including the co-culture of HepG2 with HUVEC cells, can increase the performance of the created liver model.	[181]
Extrusion, microfluidics	HepG2/C3A	GelMA/ECM	The viability and drug toxicity screening was performed on hepatic spheroids embedded in an ECM-based hydrogel scaffoldand placed in a microfluidic device.	[182]
Extrusion	Primary human hepatocyte/HSC/HUVEC cell	NovoGel 2.0 Hydrogel	Trovafloxacin utilized to assess the hepatocyte toxicity in 3D-microscale liver cells in order to replicate the tissue-level DILI.	[8]
Inkjet/Microfluidic chip	HepG2/U-251 cell (human glioblastoma cell line)	Alginate sodium/PDMS	Microchip-based assessment of the drug metabolism and diffusion in microscale constructs fabricated using co-printed HepG2 and U-251 cells, where cell printing was spatially regulated.	[183]
(Photocuring- based) DLP	hiPSC-HPCs/HUVECs/ ADSCs	GelMA, GMHA	The 3D built model offered an environment for hiPSC-HPCs that increased not only their anabolic and catabolic activities, but also the critical CYP expression levels, mature gene expression profiles, and drug metabolism capability.	[121]
Extrusion	Primary rat hepatocytes/ HUVECs/HLFs	Collagen (cells laden hydrogel)/PCL (framework material)	The printing of three types of cells in natural hydrogels, including hepatocytes, HUVEC cells, and HLF cells, and the use of PCL as a framework material, resulted in the development of a 3D structure with good mechanical characteristics and capillary networks.	[36]
Inkjet/microfluidic	HepG2/C3A	GelMA	The results demonstrated similarity of a 3D organ-on-a-chip structure's function with those of *in vivo* and other in vitro systems.	[184]
Extrusion	HepG2	Alginate/Gelatin/Fibrinogen	The microscopic assay demonstrated high viability of cells loaded in an alginate/gelatin/fibrinogen hydrogel. The effect of chemotherapy drugs such as 5-Fluorouracil, mitomycin and their combination on hepatoma cells could be investigated and showed a significant change compared to a 2D model.	[185]
Extrusion	hiPSC- HLCs/HESC	Alginate	The development of embryonic stem cells and hiPSC into hepatocyte-like cells was studied using a valve-based bioprinted construct.	[186]
CRMS	ADSCs/hepatocytes	Fibrin/PLGA		[187]

(*continued on next page*)

Table 2 (*continued*)

Bioprinting strategy	Bioink		Outcomes	Ref.
	Cells	Hydrogel materials		
			The construct transplantation to nude mice for the *in vivo* evaluation of the liver function revealed that ADSC/hepatocyte mixes had significantly increased ALB and TP secretion abilities. Furthermore, ADSCs could successfully differentiate to endothelial cells after the influence of hepatic growth factors produced by hepatocytes.	
Extrusion	HepG2/NIH3T3	GelMA	Direct-write bioprinted HepG2 cells in a GelMA hydrogel with adequate mechanical characteristics remained viable for more than a week.	[188]
Inkjet/microfluidics	HepG2/HUVEC	Fibronectin-gelatin	The metabolic and protein secretory function of hepatocytes in a hierarchical co-culture was assessed, and the cell toxicity with troglitazone was measured using a live/dead assay during the fabrication of multilayered micro-tissues.	[112]

Abbreviations. aHSC – activated hepatic stellate cells, PMHs – primary mouse hepatocytes, dECM – decellularized extracellular matrix, HUVEC – human umbilical vein endothelial cells, MSCs - mesenchymal stem cells, HFF- human foreskin fibroblast, algMC - alginate and methylcellulose, HCC – hepatocellular carcinoma, DLP – digital light processing, hiHep – human-induced hepatocytes, GelMA – gelatin methacryloyl, PEG – polyethylene glycol, ECs – endothelial cells, ICC - intrahepatic cholangiocarcinoma, hiPSC – human induced pluripotent stem cell, HSCs – hepatic stellate cells, KCs – kupffer cells, hLdECM – human Lung decellularized ECM, SLA – stereolithography, TG hydrogel - transglutaminase hydrogel, PLA - poly(lactic acid), PVA – Poly(vinyl alcohol), UCB-MSC – Umbilical cord blood-MSC, GA – galactosylated alginate, ASGPR – asialoglycoprotein receptor, BMMSCs – bone marrow-derived mesenchymal stem cells, PCL – polycaprolactone, miHeps – mouse-induced hepatocyte-like cells, CNCs – cellulose nanocrystals, hASCs – human adipose derived Stem cells, PDMS –polydimethylsiloxane, hiPSC-HPCs - hiPSC-derived hepatic progenitor cells, ADSCs - adipose derived-stem cells, GMHA – glycidal methacrylate-hyaluronic acid, HLFs – human lung fibroblasts, hiPSC- HLCs – human induced pluripotent stem cell – derived hepatocyte like cells, HESC – human embryonic stem cell, CRMS – combined rotational mold system, ADSCs –adipose-derived stem cells.

and access to a donor are major challenging subjects. To overcome such obstacles, PHH can be replaced by other candidates, such as hepatic lines or stem cell-derived hepatocyte-like cells. However, these alternatives have both advantages and disadvantages compared to PHHs [133].

Different studies have revealed that some hepatoma cell lines such as Huh7 [134], HepG2 [58,135,136] and HepaRG [79,137,138] are able to perform many PHH functions. These similarities result in a broad application of these cells in bioprinting. As a drawback, the metabolic activity of these hepatoma cells is not comparable to PHH [139,140], although some approaches to improve their metabolic activity have been described [133,141,142]. The only exception reported is HepaRG cells, which show high metabolic activity [143]. However, some researchers are still skeptical about the HepaRG applicability for drug toxicity screening. This cell line showed a lower sensitivity in response to liver toxic drugs when compared to HepG2 cells [144].

Taken together, HepaRG cells, known as hepatic progenitor cells, could be the most promising cell line in liver bioprinting because of their ability to differentiate into functional hepatocytes and express phase I and II metabolic enzymes. Also, these cells can generate the bile canaliculi structures. Moreover, bioprinted HepaRG cells were able to function *in vivo* and improve the survival rate after transplantation into mice, that presumes their great potential in the liver regeneration [79].

Stem cell technology has shown great promise in the development of drug screening, personalized medicine, and disease modeling [145–147]. Hepatocytes can be derived from multiple stem cell types, including embryonic stem cells (ESCs), iPSCs, MSCs, fetal liver cells, and human adult liver SCs/hepatic liver stem cells(41). Among these cell sources, hepatocytes derived from ESC, iPSC and adipose-MSC have been successfully used in 3D bioprinting [18,67,136,148,149].

Some practical advantages of iPSC compared to ESC make them a promising source in the field of tissue engineering, including the ability to self-renewal in the long term and large-scale setting. They can differentiate to all cell types and can be generated from fully differentiated cells from healthy donors. Hence, unlike ESC, iPSC can overcome the immune rejection issues, and there are no ethical issues in their clinical application [150]. In the study [18], the authors printed hiPSC-hepatic progenitor cells and supportive cells embedded in GMHA and GelMA, using a valve-based bioprinting technique. The hepatic lobule structure was recapitulated, and the obtained data demonstrated its advanced morphological organization, increased liver-specific gene expression levels, and the metabolic product secretion in bioprinted hiPSC- derived hepatic progenitor cells (hiPSC-HPCs), compared to a 2D culture and a 3D HPC-only model (with no supporting cells) [18].

However, the current methods face many challenges, such as heterogeneity in differentiated cells and inadequate functionality of HLCs. The current developments in differentiation methods have resolved many problems in this field, including those related to 3D culturing conditions, co-culture with non-parenchymal cells, tissue microfabrication, micro-fluid flow systems, defined media and cell encapsulation approaches [151]. For example, microfluidic systems and microchips are the best devices to mimic the *in vivo* cell environment, they also contain a blood flow equivalent, which transports oxygen and nutrients and excrete waste materials. This approach was used for hiPSC differentiation and demonstrated that microfluidic biochips were promising in providing the required microenvironment for hepatocyte differentiation [152]. Another approach to improve iPSCs differentiation is fabrication of 3D structures, specifically organoids, which repeat some of the *in vivo* functions and mimic many cellular interactions in an organ. Merging co-culture systems with the 3D-culture approach has improved the maturation of HLCs [153].

Then, application of small molecules also enhanced the differentiation efficiency. The most important examples are CHIR99021 which induces the WNT/β-catenin pathway and Ly294002, which is synergized with the Activin/Nodal pathway during the initial days of differentiation [154–156]. During the endoderm specification, retinoic acid can enhance differentiation of stem cells into the hepatic lineage [157].

4. Vascularization challenges in liver bioprinting

Liver tissue engineering aims at developing tissue-specific compositions consisting of the ECM, parenchymal and non-parenchymal cells, as well as the organ microarchitecture including the vascular system, bile ducts and interconnected channels [189,190]. The vascular network formation is one of crucial challenges in tissue engineering. Engineered 3D tissue constructs must be small with low diameters, about 100–200 μm, for the oxygen and nutrient supply and to avoid formation of a necrotic core [12,191]. Many researchers have used the bioprinting technique for patterning the vasculature, including microchannel embedding [12,192], application of angiogenic growth factors or

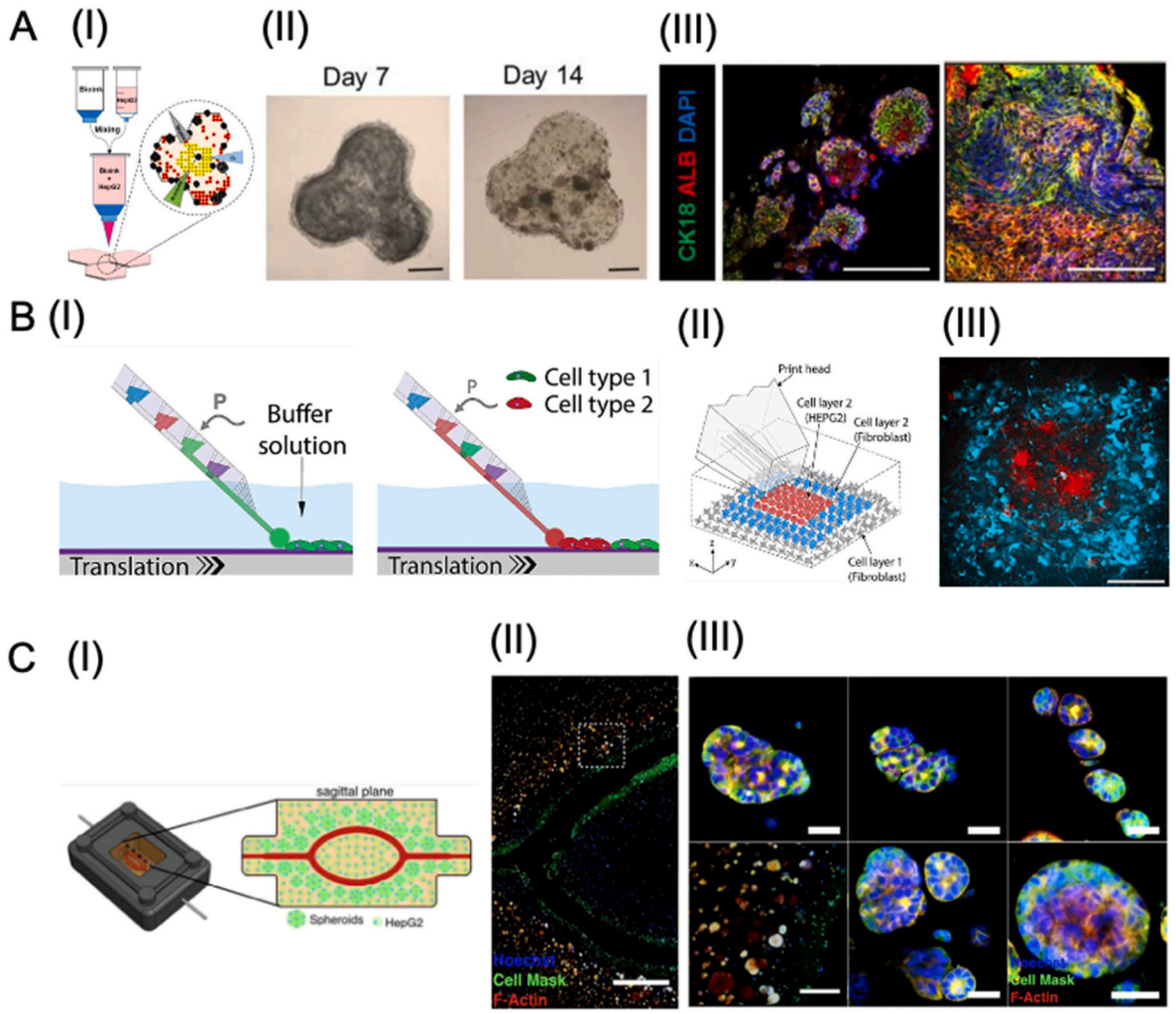

Fig. 3. Examples of recent achievements in the liver tissue bioprinting:
A – Hexagonal HepG2-based bioprinted construct: I – scheme; II –morphology; III – bioprinted hepatic construct (cytokeratin 18 (green) and albumin (red), scale bar = 250 μm). The images were adapted and changed from Ref. [76] according to http://creativecommons.org/licenses/by/4.0/. B – Microfluidic bioprinting of the HepG2-and 3T3-containing construct: I –principle; II –model: III –construct 24h after printing (fibroblasts (blue), HepG2 (red)). The images were adapted and changed from Ref. [163] according to http://creativecommons.org/licenses/by/4.0/. C – Vascularized liver construct fabricated using bioprinting and microfluidics: I – scheme; II – cross-section of the 15 d tissue construct (nuclei (blue), cellular membrane (green) and F-actin (red), scale bar = 2 mm); III – morphology of the 15 d perfused HepG2 spheroids (nuclei (blue), cellular membrane (green) and F-actin (red)). The images were adapted and changed from Ref. [171] according to http://creativecommons.org/licenses/by/4.0/. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

endothelial cells [193], sacrificial polymers to fabricate the microchannel networks [194], and bioprinting of the vascular system using direct cell patterning [195].

It has been revealed that the construction of cell-laden constructs *via* designed microchannels can increase the cell viability and differentiation capacity compared to the constructs without microchannels [12]. One of important challenges that must be considered is the small size of capillaries for printing. An elaborate predefined design is needed for the interconnection of microchannels through the angiogenesis [196]. Hence, to generate living constructs in a larger size, microchannels embedding and a predefined design are required [197].

5. Conclusions

3D bioprinting can be used as a developing tool to improve a biological substitute. The bioprinted organs should demonstrate the physiological functions, biomechanical properties, and vascular networks comparable to those of the natural organ. To better mimic these properties, application of more consistent cells, materials and precise bioprinters can be considered as three sides of a triangle in the development of a bioprinting technology (Fig. 4). However, since bioprinting is an evolving technology, there are standards and adjusted materials, cells and bioprinters which are still not globally accepted.

In the case of bioprinters, the 4D bioprinting technology has been developed as a powerful platform, which added the “time value” to 3D bioprinting. This technique uses stimuli-response (smart) biomaterials which are capable of changing the shape of a construct over time, that results in engineering more complex constructs. The 4D bioprinting approach may result in designing more satisfactory microenvironments compared to conventional 3D bioprinting systems. In terms of the cell sources, stem cell-derived HLCs, rather than primary hepatocytes, are promising for the construction of 3D structures. However, some issues need to be considered when these cells are used for 3D bioprinting, such as optimization of the cellular microenvironment and its mechanical stability, in order to recapitulate the *in vivo* environment. Moreover, 3D printed liver structures with individual cells have limitations due to the inability to mimic the natural liver cell density and functional properties. To address this challenge, bioprinting of spheroids has been applied

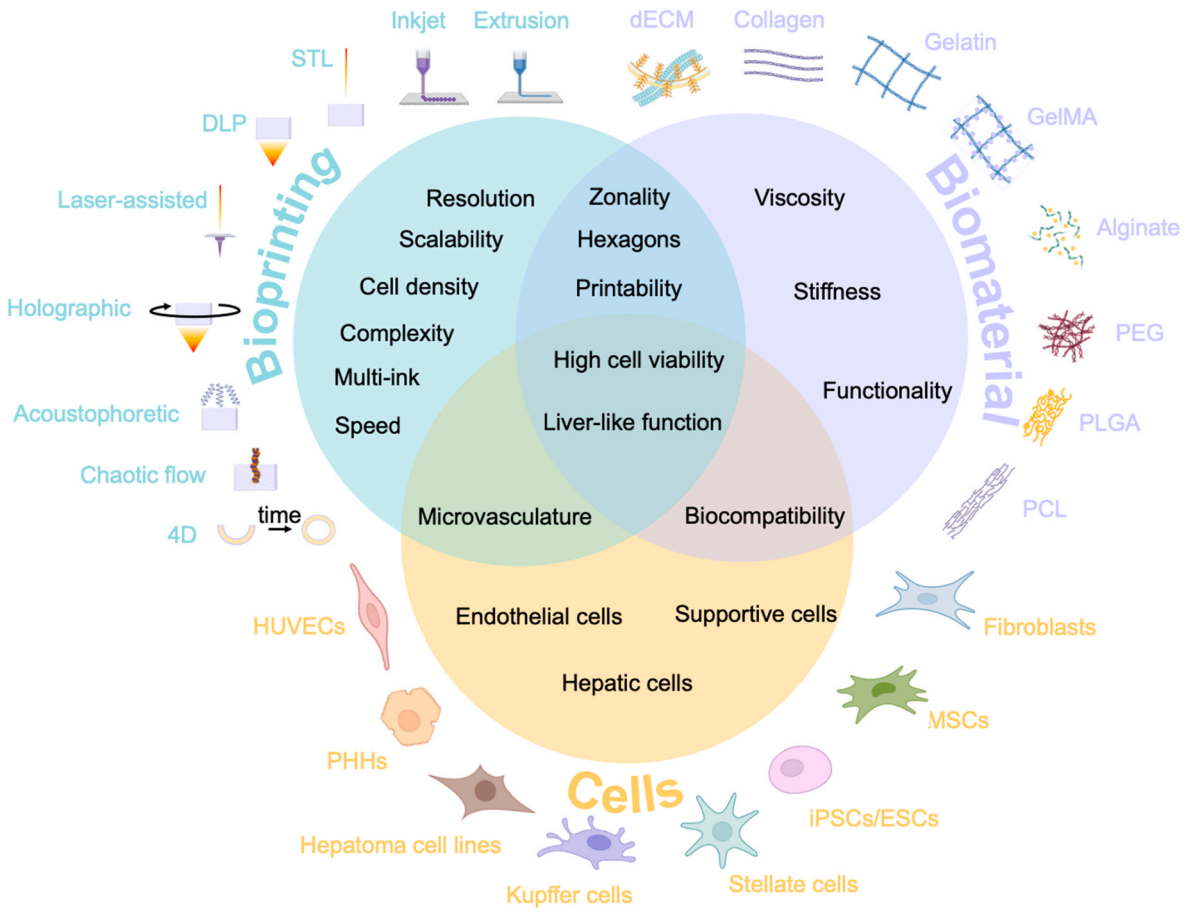

Fig. 4. The concept of the liver tissue engineering based on the Triade 'Cells-Biomaterial-Bioprinting'. Inside the diagram, there are blocks and features necessary in fabricating a liver bioequivalent. Outside the diagram, examples (yellow – cells, blue – bioprinting, and violet – biomaterial) are given.
dECM – decellularized extracellular matrix; DLP – digital light processing; ESCs – embryonic stem cells; GelMA – gelatin methacrylamine; HUVECs – human endothelial umbilical vein endothelial cells; iPSCs –induced pluripotent stem cells; MSCs – mesenchymal stromal cells; PCL – polycaprolactone; PEG – polyethylene glycol; PLGA – poly(lactic-co-glycolic acid); PHHs – primary human hepatocytes; STL – stereolitography. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

in many studies. The most important element for efficient bioprinting is bioinks. Bioinks should meet several basic requirements, such as the mechanical, biological, and rheological factors. Furthermore, an appropriate stiffness is required to maintain the entire structure over a long period of time. Balancing between the bio-printability and bio-functionality of bioinks ought to be considered in new research projects. On the other hand, using single biomaterials cannot provide the high printability and biological functionality at the same time. A comprehensive understanding of the liver ECM is necessary to compose multicomponent bioinks similar to the liver ECM structure. In this case, many liver ECM components are involved in the morphology, behavior, and function of cells through the direct interactions with liver cells. However, it is difficult to recapitulate the interactions of such a dynamic *in vivo* complex between the liver ECM and cells *via* a single natural biomaterial. Many studies have considered multicomponent bioinks in combination with the liver dECM. It is necessary to propose and validate a standard decellularization protocol that can completely remove the cellular components and preserve the ECM elements. Furthermore, using novel crosslinking approaches in order to rapidly prepare biocompatible printable multicomponent bioinks is essential. For example, the photo-crosslinking method is widely used in tissue engineering, as it is rapid, controllable, and valid in many printing techniques. To avoid any undesirable impact on cells, it is essential to utilize the photo-crosslinking method that employs visible (not UV) light.

In conclusion, although the 3D bioprinting technique is still in its infancy, it has a great potential to overcome the many challenges related to the construction of complex tissues in order to mimic the natural conditions in a human body. Bioinks composed of new biomaterials in combination with dECM could be ideal bioinks for the bioprinting strategies. Furthermore, using a 3D structure as a substitute for a monolayer culture can address the limitations in liver tissue engineering.

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Authors' contribution

Z.H. drafted the manuscript, edited, and generated the concept of manuscript. P.P. and P.B. designed the figures. P.B., A.P., V.F., H.S., and A.S. wrote their subtitles and edited their sections. P.T. and M.V. generated the concept and involved in writing and editing and proofing the manuscript. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Z. Heydari, M. Najimi, H. Mirzaei, A. Shpichka, M. Ruoss, Z. Farzaneh, et al., Tissue engineering in liver regenerative medicine: insights into novel translational technologies, 2 9, 2020, p. 304.

[2] T. Agarwal, B. Subramanian, T.K. Maiti, Liver tissue engineering: challenges and opportunities, ACS Biomater. Sci. Eng. 5 (9) (2019) 4167–4182.

[3] A.A. Palakkan, D.C. Hay, A.K. Pr, K. Tv, J.A. Ross, Liver tissue engineering and cell sources: issues and challenges, Liver Int. 33 (5) (2013) 666–676.

[4] A. Damania, E. Jain, A. Kumar, Advancements in in vitro hepatic models: application for drug screening and therapeutics, Hepatol. Int. 8 (1) (2014) 23–38.

[5] T. Agarwal, D. Banerjee, R. Konwarh, T. Esworthy, J. Kumari, V. Onesto, et al., Recent advances in bioprinting technologies for engineering hepatic tissue, Mater. Sci. Eng. C 123 (2021), 112013.

[6] C. Kryou, V. Leva, M. Chatzipetrou, I. Zergioti, Bioprinting for liver transplantation, Bioengineering 6 (4) (2019) 95.

[7] A. Faulkner-Jones, C. Fyfe, D.-J. Cornelissen, J. Gardner, J. King, A. Courtney, et al., Bioprinting of human pluripotent stem cells and their directed differentiation into hepatocyte-like cells for the generation of mini-livers in 3D, Biofabrication 7 (4) (2015), 044102.

[8] D.G. Nguyen, J. Funk, J.B. Robbins, C. Crogan-Grundy, S.C. Presnell, T. Singer, et al., Bioprinted 3D primary liver tissues allow assessment of organ-level response to clinical drug induced toxicity in vitro, PLoS One 11 (7) (2016), e0158674.

[9] H. Kizawa, E. Nagao, M. Shimamura, G. Zhang, H. Torii, B. Reports, Scaffold-free 3D bio-printed human liver tissue stably maintains metabolic functions useful for drug discovery, Biochem. BiophyS. Rep. 10 (2017) 186–191.

[10] L.M. Ott, K. Ramachandran, L. Stehno-Bittel, An automated multiplexed hepatotoxicity and CYP induction assay using HepaRG cells in 2D and 3D, SLAS DISCOVERY: Adv. Life Sci. R&D 22 (5) (2017) 614–625.

[11] F.J. O'brien, Biomaterials & scaffolds for tissue engineering, Mater. Today 14 (3) (2011) 88–95.

[12] L.E. Bertassoni, M. Cecconi, V. Manoharan, M. Nikkhah, J. Hjortnaes, A. L. Cristino, et al., Hydrogel bioprinted microchannel networks for vascularization of tissue engineering constructs, 13 14, 2014, pp. 2202–2211.

[13] T.J. Hinton, Q. Jallerat, R.N. Palchesko, J.H. Park, M.S. Grodzicki, H.-J. Shue, et al., Three-dimensional printing of complex biological structures by freeform reversible embedding of suspended hydrogels, Sci. Adv. 1 (9) (2015), e1500758.

[14] D.T. Carvalho, T. Feijão, M.I. Neves, R.M. da Silva, C.C. Barrias, Directed self-assembly of spheroids into modular vascular beds for engineering large tissue constructs, Biofabrication 13 (3) (2021), 035008.

[15] G. Gao, J.H. Lee, J. Jang, D.H. Lee, J.S. Kong, B.S. Kim, et al., Tissue engineered bio-blood-vessels constructed using a tissue-specific bioink and 3D coaxial cell printing technique: a novel therapy for ischemic disease, Adv. Funct. Mater. 27 (33) (2017), 1700798.

[16] W. Liu, Z. Zhong, N. Hu, Y. Zhou, L. Maggio, A.K. Miri, et al., Coaxial extrusion bioprinting of 3D microfibrous constructs with cell-favorable gelatin methacryloyl microenvironments, 2 10, 2018, 024102.

[17] J. Sarkar, S.C. Kamble, N.C.J.C. Kashikar, Polymeric Bioinks for 3D Hepatic Printing, 1 3, 2021, pp. 164–181.

[18] X. Ma, X. Qu, W. Zhu, Y.-S. Li, S. Yuan, H. Zhang, et al., Deterministically patterned biomimetic human iPSC-derived hepatic model via rapid 3D bioprinting, 8 113, 2016, pp. 2206–2211.

[19] T.-Y. Kang, J.M. Hong, J.W. Jung, H.-W. Kang, D.-W. Cho, Construction of large-volume tissue mimics with 3D functional vascular networks, PLoS One 11 (5) (2016), e0156529.

[20] S.V. Murphy, Atala AJNb, 3D bioprinting of tissues and organs 32, 2014, pp. 773–785.

[21] M. Hospodiuk, M. Dey, D. Sosnoski, I.T. Ozbolat, The bioink: a comprehensive review on bioprintable materials, Biotechnol. Adv. 35 (2) (2017) 217–239.

[22] R. Fan, M. Piou, E. Darling, D. Cormier, J. Sun, J.J. Wan, Bio-printing cell-laden Matrigel–agarose constructs, J. Biomater. Appl. 31 (5) (2016) 684–692.

[23] F.P. Melchels, J. Feijen, D.W. Grijpma, A review on stereolithography and its applications in biomedical engineering, Biomaterials 31 (24) (2010) 6121–6130.

[24] D.-Y. Lee, H. Lee, Y. Kim, S.Y. Yoo, W.-J. Chung, G. Kim, Phage as versatile nanoink for printing 3-D cell-laden scaffolds, Acta Biomater. 29 (2016) 112–124.

[25] F. Liu, C. Liu, Q. Chen, Q. Ao, X. Tian, J. Fan, et al., Progress in organ 3D bioprinting 4, 2018, 1.

[26] X.J.M. Wang, Advanced polymers for three-dimensional (3D) organ bioprinting, 12 10, 2019, p. 814.

[27] J.H. Chung, S. Naficy, Z. Yue, R. Kapsa, A. Quigley, S.E. Moulton, et al., Bio-ink properties and printability for extrusion printing living cells, Biomater. Sci. 1 (7) (2013) 763–773.

[28] F. Liu, Q. Chen, C. Liu, Q. Ao, X. Tian, J. Fan, et al., Natural polymers for organ 3D bioprinting, 11 10, 2018, p. 1278.

[29] A. Schwab, R. Levato, M. D'Este, S. Piluso, D. Eglin, J. Malda, Printability and shape fidelity of bioinks in 3D bioprinting, Chem. Rev. 120 (19) (2020) 11028–11055.

[30] K.-H. Lee, J. Lee, S.-H. Lee, 3D liver models on a microplatform: well-defined culture, engineering of liver tissue and liver-on-a-chip, Lab Chip 15 (19) (2015) 3822–3837.

[31] S.N. Bhatia, G.H. Underhill, K.S. Zaret, I.J. Fox, Cell and tissue engineering for liver disease, Sci. Transl. Med. 6 (245) (2014), 245sr2-sr2.

[32] S.S. Ng, A. Xiong, K. Nguyen, M. Masek, D.Y. No, M. Elazar, et al., Long-term culture of human liver tissue with advanced hepatic functions, Jci Insight 2 (11) (2017).

[33] J. Gopinathan, I. Noh, Recent trends in bioinks for 3D printing, Biomater. Res. 22 (1) (2018) 1–15.

[34] N. Siddiqui, S. Asawa, B. Birru, R. Baadhe, S. Rao, PCL-based composite scaffold matrices for tissue engineering applications, Mol. Biotechnol. 60 (7) (2018) 506–532.

[35] R. Grant, D.C. Hay, A. Callanan, A drug-induced hybrid electrospun poly-capro-lactone: cell-derived extracellular matrix scaffold for liver tissue engineering, Tissue Eng. A 23 (13–14) (2017) 650–662.

[36] J.W. Lee, Y.-J. Choi, W.-J. Yong, F. Pati, J.-H. Shim, K.S. Kang, et al., Development of a 3D cell printed construct considering angiogenesis for liver tissue engineering, Biofabrication 8 (1) (2016), 015007.

[37] N. Ashammakhi, S. Ahadian, C. Xu, H. Montazerian, H. Ko, R. Nasiri, et al., Bioinks and bioprinting technologies to make heterogeneous and biomimetic tissue constructs 1, 2019, 100008.

[38] M. Sheridan, L. Shea, M. Peters, D. Mooney, Bioabsorbable polymer scaffolds for tissue engineering capable of sustained growth factor delivery, J. Contr. Release 64 (1–3) (2000) 91–102.

[39] J. Kasuya, R. Sudo, R. Tamogami, G. Masuda, T. Mitaka, M. Ikeda, et al., Reconstruction of 3D stacked hepatocyte tissues using degradable, microporous poly (d, l-lactide-co-glycolide) membranes, Biomaterials 33 (9) (2012) 2693–2700.

[40] U.J. Park, W. Jeong, S.Y. Kwon, Y. Kim, K. Choi, H.T. Kim, et al., Fabrication of a novel absorbable vascular anastomosis device and testing in a pig liver transplantation model, Ann. Biomed. Eng. 47 (4) (2019) 1063–1077.

[41] L. Ma, Y. Wu, Y. Li, A. Aazmi, H. Zhou, B. Zhang, et al., Current Advances on 3D-Bioprinted Liver Tissue Models, 24 9, 2020, 2001517.

[42] K. Christensen, C. Xu, W. Chai, Z. Zhang, J. Fu, Y. Huang, et al., Freeform inkjet printing of cellular structures with bifurcations, Biotechnol. Bioeng. 112 (5) (2015) 1047–1055.

[43] W. Jia, P.S. Gungor-Ozkerim, Y.S. Zhang, K. Yue, K. Zhu, W. Liu, et al., Direct 3D bioprinting of perfusable vascular constructs using a blend bioink 106, 2016, pp. 58–68.

[44] S.N. Pawar, K.J. Edgar, Alginate derivatization: a review of chemistry, properties and applications, Biomaterials 33 (11) (2012) 3279–3305.

[45] K.Y. Lee, D.J. Mooney, Alginate: properties and biomedical applications, Prog. Polym. Sci. 37 (1) (2012) 106–126.

[46] M. Stanton, J. Samitier, S. Sanchez, Bioprinting of 3D hydrogels, Lab Chip 15 (15) (2015) 3111–3115.

[47] P. Gentile, K. McColgan-Bannon, N.C. Gianone, F. Sefat, K. Dalgarno, A. M. Ferreira, Biosynthetic PCL-graft-collagen bulk material for tissue engineering applications, Materials 10 (7) (2017) 693.

[48] E.O. Osidak, V.I. Kozhukhov, M.S. Osidak, S.P. Domogatsky, Collagen as bioink for bioprinting: a comprehensive review, Int. J. Bioprint. 6 (3) (2020).

[49] J.W. Lee, Y.-J. Choi, W.-J. Yong, F. Pati, J.-H. Shim, K.S. Kang, et al., Development of a 3D cell printed construct considering angiogenesis for liver tissue engineering, Biofabrication 8 (1) (2016), 015007.

[50] A. Mazzocchi, M. Devarasetty, R. Huntwork, S. Soker, A. Skardal, Optimization of collagen type I-hyaluronan hybrid bioink for 3D bioprinted liver microenvironments, Biofabrication 11 (1) (2018), 015003.

[51] A. Mazzocchi, M. Devarasetty, R. Huntwork, S. Soker, A. Skardal, Optimization of collagen type I-hyaluronan hybrid bioink for 3D bioprinted liver microenvironments, Biofabrication 11 (1) (2018), 015003.

[52] F. Rodriguez-Pascual, D.A. Slatter, Collagen cross-linking: insights on the evolution of metazoan extracellular matrix, Sci. Rep. 6 (1) (2016) 1–7.

[53] H. Stratesteffen, M. Köpf, F. Kreimendahl, A. Blaeser, S. Jockenhoevel, H. Fischer, GelMA-collagen blends enable drop-on-demand 3D printablility and promote angiogenesis, Biofabrication 9 (4) (2017), 045002.

[54] X. Wang, X. Yu, Y. Yan, R.J. Zhang, The Japanese Society for Biomaterials, Biomaterials TASf, Biomaterials tKSf. Liver tissue responses to gelatin and gelatin/chitosan gels, JoBMRPAAOJoTSfB 87 (1) (2008) 62–68.

[55] A. Tripathi, J.S. Melo, Preparation of a sponge-like biocomposite agarose–chitosan scaffold with primary hepatocytes for establishing an in vitro 3D liver tissue model, RSC Adv. 5 (39) (2015) 30701–30710.

[56] W.L. Ng, W.Y. Yeong, Naing MW, Polyelectrolyte gelatin-chitosan hydrogel optimized for 3D bioprinting in skin tissue engineering, Int. J. Bioprint. 2 (1) (2016).

[57] N.S. Bhise, V. Manoharan, S. Massa, A. Tamayol, M. Ghaderi, M. Miscuglio, et al., A liver-on-a-chip platform with bioprinted hepatic spheroids, 1 8, 2016, 014101.

[58] T. Billiet, E. Gevaert, T. De Schryver, M. Cornelissen, P. Dubruel, The 3D printing of gelatin methacrylamide cell-laden tissue-engineered constructs with high cell viability, Biomaterials 35 (1) (2014) 49–62.

[59] J. Baier Leach, K.A. Bivens, C.W. Patrick Jr., C.E. Schmidt, Photocrosslinked hyaluronic acid hydrogels: natural, biodegradable tissue engineering scaffolds, Biotechnol. Bioeng. 82 (5) (2003) 578–589.

[60] D. Kim, M. Kim, J. Lee, J. Jang, Review on multicomponent hydrogel bioinks based on natural biomaterials for bioprinting 3D liver tissues, Front. Bioeng. Biotechnol. 10 (2022).

[61] R.P. Roopesh, S. Muthusamy, S. Velayudhan, A. Sabareeswaran, P.R.A. Kumar, High-throughput production of liver parenchymal microtissues and enrichment of organ-specific functions in gelatin methacrylamide microenvironment, Biotechnol. Bioeng. (2022).
[62] J. Cui, H. Wang, Q. Shi, T. Sun, Q. Huang, T. Fukuda, Multicellular co-culture in three-dimensional gelatin methacryloyl hydrogels for liver tissue engineering, Molecules 24 (9) (2019) 1762.
[63] V.Z. Beachley, M.T. Wolf, K. Sadtler, S.S. Manda, H. Jacobs, M.R. Blatchley, et al., Tissue matrix arrays for high-throughput screening and systems analysis of cell function, 12 12, 2015, pp. 1197–1204.
[64] K. Dzobo, K.S.C.M. Motaung, AJIjoms Adesida, Recent trends in decellularized extracellular matrix bioinks for 3D printing: an updated review, Int. J. Mol. Sci. 20 (18) (2019) 4628.
[65] M. Ruoß, M. Vosough, A. Königsrainer, S. Nadalin, S. Wagner, S. Sajadian, et al., Towards improved hepatocyte cultures: progress and limitations 138, 2020, 111188.
[66] D. Choudhury, H.W. Tun, T. Wang, Naing MWJTib, Organ-derived decellularized extracellular matrix: a game changer for bioink manufacturing? Trends Biotechnol. 36 (8) (2018) 787–805.
[67] C. Yu, X. Ma, W. Zhu, P. Wang, K.L. Miller, J. Stupin, et al., Scanningless and continuous 3D bioprinting of human tissues with decellularized extracellular matrix, 194, 2019, pp. 1–13.
[68] Q. Mao, Y. Wang, Y. Li, S. Juengpanich, W. Li, M. Chen, et al., Fabrication of liver microtissue with liver decellularized extracellular matrix (dECM) bioink by digital light processing (DLP) bioprinting, Mater. Sci. Eng. C 109 (2020), 110625.
[69] J. Sarkar, S.C. Kamble, N.C. Kashikar, Polymeric bioinks for 3D hepatic printing, Chemistry 3 (1) (2021) 164–181.
[70] X. Ma, C. Yu, P. Wang, W. Xu, X. Wan, C.S.E. Lai, et al., Rapid 3D bioprinting of decellularized extracellular matrix with regionally varied mechanical properties and biomimetic microarchitecture, Biomaterials 185 (2018) 310–321.
[71] G. Gao, J.H. Lee, J. Jang, D.H. Lee, J.S. Kong, B.S. Kim, et al., Tissue engineered bio-blood-vessels constructed using a tissue-specific bioink and 3D coaxial cell printing technique: a novel therapy for ischemic disease, Adv. Funct. Mater. 27 (33) (2017), 1700798.
[72] J. Jang, T.G. Kim, B.S. Kim, S.-W. Kim, S.-M. Kwon, D.-W. Cho, Tailoring mechanical properties of decellularized extracellular matrix bioink by vitamin B2-induced photo-crosslinking, Acta Biomater. 33 (2016) 88–95.
[73] H. Kim, B. Kang, X. Cui, S.H. Lee, K. Lee, D.W. Cho, et al., Light-activated decellularized extracellular matrix-based bioinks for volumetric tissue analogs at the centimeter scale, Adv. Funct. Mater. 31 (32) (2021), 2011252.
[74] W. Kim, H. Lee, J. Lee, A. Atala, J.J. Yoo, S.J. Lee, et al., Efficient myotube formation in 3D bioprinted tissue construct by biochemical and topographical cues, Biomaterials 230 (2020), 119632.
[75] M.K. Kim, W. Jeong, S.M. Lee, J.B. Kim, S. Jin, H.-W. Kang, Decellularized extracellular matrix-based bio-ink with enhanced 3D printability and mechanical properties, Biofabrication 12 (2) (2020), 025003.
[76] H.K. Kang, M. Sarsenova, D.-H. Kim, M.S. Kim, J.Y. Lee, E.-A. Sung, et al., Establishing a 3D in vitro hepatic model mimicking physiologically relevant to in vivo state, 5 10, 2021, p. 1268.
[77] E. Goulart, L.C. de Caires-Junior, K.A. Telles-Silva, B.H.S. Araujo, S.A. Rocco, M. Sforca, et al., 3D bioprinting of liver spheroids derived from human induced pluripotent stem cells sustain liver function and viability in vitro, 12, 2019, 015010, 1.
[78] D.G. Nguyen, J. Funk, J.B. Robbins, C. Crogan-Grundy, S.C. Presnell, T. Singer, et al., Bioprinted 3D primary liver tissues allow assessment of organ-level response to clinical drug induced toxicity in vitro, 11, 2016, e0158674, 7.
[79] H. Yang, L. Sun, Y. Pang, D. Hu, H. Xu, S. Mao, et al., Three-dimensional bioprinted hepatorganoids prolong survival of mice with liver failure, 70, 2021, pp. 567–574, 3.
[80] D. Kang, G. Ahn, D. Kim, H.-W. Kang, S. Yun, W.-S. Yun, et al., Pre-set extrusion bioprinting for multiscale heterogeneous tissue structure fabrication, 10, 2018, 035008, 3.
[81] M. Cuvellier, F. Ezan, H. Oliveira, S. Rose, J.-C. Fricain, S. Langouët, et al., 3D culture of HepaRG cells in GelMa and its application to bioprinting of a multicellular hepatic model 269, 2021, 120611.
[82] M. Matsusaki, K. Sakaue, K. Kadowaki, Akashi MJAhm, Three-dimensional human tissue chips fabricated by rapid and automatic inkjet cell printing, Adv. Healthc. Mater. 2 (4) (2013) 534–539.
[83] K. Hölzl, S. Lin, L. Tytgat, S. Van Vlierberghe, L. Gu, A. Ovsianikov, Bioink properties before, during and after 3D bioprinting, Biofabrication 8 (3) (2016), 032002.
[84] T. Xu, J. Jin, C. Gregory, J.J. Hickman, T. Boland, Inkjet printing of viable mammalian cells, Biomaterials 26 (1) (2005) 93–99.
[85] Y.B. Kang, T.R. Sodunke, J. Lamontagne, J. Cirillo, C. Rajiv, M.J. Bouchard, et al., Liver sinusoid on a Chip: Long-Term Layered Co-Culture of Primary Rat Hepatocytes and Endothelial Cells in Microfluidic Platforms 112, 2015, pp. 2571–2582 (12).
[86] L. Koch, A. Deiwick, A. Franke, K. Schwanke, A. Haverich, R. Zweigerdt, et al., Laser bioprinting of human induced pluripotent stem cells—the effect of printing and biomaterials on cell survival, pluripotency, and differentiation, 3 10, 2018, 035005.
[87] A. Karaiskou, I. Zergioti, C. Fotakis, M. Kapsetaki, D. Kafetzopoulos, Microfabrication of biomaterials by the sub-ps laser-induced forward transfer process, Appl. Surf. Sci. 208 (2003) 245–249.
[88] A. Antoshin, S. Churbanov, N. Minaev, D. Zhang, Y. Zhang, A. Shpichka, et al., LIFT-bioprinting, is it worth it? 15, 2019, e00052.
[89] V. Leva, M. Chatzipetrou, L. Alexopoulos, D.S. Tzeranis, I. Zergioti, Direct laser printing of liver cells on porous collagen scaffolds, J. Micro Nanoeng. 13 (3) (2018) 234–237.
[90] H. Quan, T. Zhang, H. Xu, S. Luo, J. Nie, X. Zhu, Photo-curing 3D printing technique and its challenges, Bioact. Mater. 5 (1) (2020) 110–115.
[91] R. Raman, B. Bhaduri, M. Mir, A. Shkumatov, M.K. Lee, G. Popescu, et al., High-Resolution Projection Microstereolithography for Patterning of Neovasculature 5, 2016, pp. 610–619 (5).
[92] C.A. Murphy, K.S. Lim, T.B. Woodfield, Next evolution in organ-scale biofabrication: bioresin design for rapid high-resolution vat polymerization, Adv. Mater. (2022), 2107759.
[93] J. Huh, Y.-W. Moon, J. Park, A. Atala, J.J. Yoo, S.J. Lee, Combinations of photoinitiator and UV absorber for cell-based digital light processing (DLP) bioprinting, Biofabrication 13 (3) (2021), 034103.
[94] Z. Wang, R. Abdulla, B. Parker, R. Samanipour, S. Ghosh, K. Kim, A simple and high-resolution stereolithography-based 3D bioprinting system using visible light crosslinkable bioinks, Biofabrication 7 (4) (2015), 045009.
[95] K.S. Lim, R. Levato, P.F. Costa, M.D. Castilho, C.R. Alcala-Orozco, K.M. Van Dorenmalen, et al., Bio-Resin for High Resolution Lithography-Based Biofabrication of Complex Cell-Laden Constructs 10, 2018, 034101 (3).
[96] L. Shao, R. Hou, Y. Zhu, Y. Yao, Pre-shear bioprinting of highly oriented porous hydrogel microfibers to construct anisotropic tissues, Biomater. Sci. 9 (20) (2021) 6763–6771.
[97] G. Trujillo-de Santiago, M.M. Alvarez, M. Samandari, G. Prakash, G. Chandrabhatla, P.I. Rellstab-Sánchez, et al., Chaotic Printing: Using Chaos to Fabricate Densely Packed Micro-and Nanostructures at High Resolution and Speed 5, 2018, pp. 813–822 (5).
[98] F. Momeni, X. Liu, J.J.M. Ni, A review of 4D printing, Design 122 (2017) 42–79.
[99] Z. Ma, A.W. Holle, K. Melde, T. Qiu, K. Poeppel, V.M. Kadiri, et al., Acoustic Holographic Cell Patterning in a Biocompatible Hydrogel 32, 2020, 1904181 (4).
[100] C. Chávez-Madero, M.D. de León-Derby, M. Samandari, C.F. Ceballos-González, E. J. Bolívar-Monsalve, C. Mendoza-Buenrostro, et al., Using Chaotic Advection for Facile High-Throughput Fabrication of Ordered Multilayer Micro-and Nanostructures: Continuous Chaotic Printing 12, 2020, 035023 (3).
[101] D. Therriault, S.R. White, J.A. Lewis, Chaotic mixing in three-dimensional microvascular networks fabricated by direct-write assembly, Nat. Mater. 2 (4) (2003) 265–271.
[102] Q. Zhang, K. Zhang, G. Hu, Smart three-dimensional lightweight structure triggered from a thin composite sheet via 3D printing technique, Sci. Rep. 6 (1) (2016) 1–8.
[103] G. Villar, A.D. Graham, H.J.S. Bayley, A Tissue-like Printed Mater. 340 (6128) (2013) 48–52.
[104] N. Rezaei, I. Akbarzadeh, S. Kazemi, L. Montazeri, I. Zarkesh, N. Hossein-Khannazer, et al., Smart Mater. Regen. Med. 4 (1) (2021) 39–51.
[105] K. Schmidt, J. Berg, V. Roehrs, J. Kurreck, M.A. Al-Zeer, 3D-bioprinted HepaRG cultures as a model for testing long term aflatoxin B1 toxicity in vitro, Toxicol Rep 7 (2020) 1578–1587.
[106] H. Yang, L. Sun, Y. Pang, D. Hu, H. Xu, S. Mao, et al., Three-dimensional bioprinted hepatorganoids prolong survival of mice with liver failure, Gut 70 (3) (2021) 567–574.
[107] L. Sun, H. Yang, Y. Wang, X. Zhang, B. Jin, F. Xie, et al., Application of a 3D bioprinted hepatocellular carcinoma cell model in antitumor drug research, Front. Oncol. 10 (2020) 878.
[108] H.K. Kang, M. Sarsenova, D.-H. Kim, M.S. Kim, J.Y. Lee, E.-A. Sung, et al., Establishing a 3D in vitro hepatic model mimicking physiologically relevant to in vivo state, Cells 10 (5) (2021) 1268.
[109] E. Goulart, L.C. de Caires-Junior, K.A. Telles-Silva, B.H.S. Araujo, S.A. Rocco, M. Sforca, et al., 3D bioprinting of liver spheroids derived from human induced pluripotent stem cells sustain liver function and viability in vitro, Biofabrication 12 (1) (2019), 015010.
[110] K. Arai, T. Yoshida, M. Okabe, M. Goto, T.A. Mir, C. Soko, et al., Fabrication of 3D-culture platform with sandwich architecture for preserving liver-specific functions of hepatocytes using 3D bioprinter, J. Biomed. Mater. Res. 105 (6) (2017) 1583–1592.
[111] K. Arai, S. Iwanaga, H. Toda, C. Genci, Y. Nishiyama, M. Nakamura, Three-dimensional inkjet biofabrication based on designed images, Biofabrication 3 (3) (2011), 034113.
[112] M. Matsusaki, K. Sakaue, K. Kadowaki, M. Akashi, Three-dimensional human tissue chips fabricated by rapid and automatic inkjet cell printing, Adv. Healthc. Mater. 2 (4) (2013) 534–539.
[113] A. Bsoul, S. Pan, E. Cretu, B. Stoeber, K. Walus, Design, microfabrication, and characterization of a moulded PDMS/SU-8 inkjet dispenser for a Lab-on-a-Printer platform technology with disposable microfluidic chip, Lab Chip 16 (17) (2016) 3351–3361.
[114] A. Antoshin, S. Churbanov, N. Minaev, D. Zhang, Y. Zhang, A. Shpichka, et al., LIFT-bioprinting, is it worth it? Bioprinting 15 (2019), e00052.
[115] V. Leva, M. Chatzipetrou, L. Alexopoulos, D.S. Tzeranis, I. Zergioti, Direct laser printing of liver cells on porous collagen scaffolds, J. Micro Nanoeng. 13 (3) (2018) 234–237.
[116] B. Guillotin, A. Souquet, S. Catros, M. Duocastella, B. Pippenger, S. Bellance, et al., Laser assisted bioprinting of engineered tissue with high cell density and microscale organization, Biomaterials 31 (28) (2010) 7250–7256.
[117] L. Koch, A. Deiwick, A. Franke, K. Schwanke, A. Haverich, R. Zweigerdt, et al., Laser bioprinting of human induced pluripotent stem cells—the effect of printing and biomaterials on cell survival, pluripotency, and differentiation, Biofabrication 10 (3) (2018), 035005.

[118] R. Raman, B. Bhaduri, M. Mir, A. Shkumatov, M.K. Lee, G. Popescu, et al., High-resolution projection microstereolithography for patterning of neovasculature, Adv. Healthc. Mater. 5 (5) (2016) 610–619.
[119] Z. Wang, R. Abdulla, B. Parker, R. Samanipour, S. Ghosh, K. Kim, A simple and high-resolution stereolithography-based 3D bioprinting system using visible light crosslinkable bioinks, Biofabrication 7 (4) (2015), 045009.
[120] K.S. Lim, R. Levato, P.F. Costa, M.D. Castilho, C.R. Alcala-Orozco, K.M. Van Dorenmalen, et al., Bio-resin for high resolution lithography-based biofabrication of complex cell-laden constructs, Biofabrication 10 (3) (2018), 034101.
[121] X. Ma, X. Qu, W. Zhu, Y.-S. Li, S. Yuan, H. Zhang, et al., Deterministically patterned biomimetic human iPSC-derived hepatic model via rapid 3D bioprinting, Proc. Natl. Acad. Sci. 113 (8) (2016) 2206–2211.
[122] M. Tanaka, T. Itoh, N. Tanimizu, Miyajima A, Liver stem/progenitor cells: their characteristics and regulatory mechanisms, J. Biochem. 149 (3) (2011) 231–239.
[123] G. Levy, D. Bomze, S. Heinz, S.D. Ramachandran, A. Noerenberg, M. Cohen, et al., Long-Term Culture and Expansion of Primary Human Hepatocytes 33, 2015, pp. 1264–1271 (12).
[124] Z. Heydari, I. Zarkesh, M.-H. Ghanian, M.H. Aghdaei, S. Kotova, E. Zahmatkesh, et al., Biofabrication of Size-Controlled Liver Microtissues Incorporated with ECM-Derived Microparticles to Prolong Hepatocyte Function, 2021, pp. 1–16.
[125] Y. Chen, P.P. Wong, L. Sjeklocha, C.J. Steer, M.B. Sahin, Mature hepatocytes exhibit unexpected plasticity by direct dedifferentiation into liver progenitor cells in culture, Hepatology 55 (2) (2012) 563–574.
[126] R.N. Bhandari, L.A. Riccalton, A.L. Lewis, J.R. Fry, A.H. Hammond, S.J. Tendler, et al., Liver tissue engineering: a role for co-culture systems in modifying hepatocyte function and viability, Tissue Eng. 7 (3) (2001) 345–357.
[127] A.A. Palakkan, D.C. Hay, A.K. Pr, K. Tv, J.A. Ross, Liver tissue engineering and cell sources: issues and challenges, Liver Int. 33 (5) (2013) 666–676.
[128] A.A. Demetriou, A. Reisner, J. Sanchez, S.M. Levenson, A.D. Moscioni, J. R. Chowdhury, Transplantation of microcarrier-attached hepatocytes into 90% partially hepatectomized rats, Hepatology 8 (5) (1988) 1006–1009.
[129] A. Baze, C. Parmentier, D.F. Hendriks, T. Hurrell, B. Heyd, P. Bachellier, et al., Three-Dimensional Spheroid Primary Human Hepatocytes in Monoculture and Coculture with Nonparenchymal Cells 24, 2018, pp. 534–545 (9).
[130] Y. Yanagi, K. Nakayama, T. Taguchi, S. Enosawa, T. Tamura, K. Yoshimaru, et al., In vivo and ex vivo Methods of Growing a Liver Bud Through Tissue Connection 7, 2017, pp. 1–15 (1).
[131] L.M. Norona, D.G. Nguyen, D.A. Gerber, S.C. Presnell, M. Mosedale, P.B. Watkins, Bioprinted liver provides early insight into the role of Kupffer cells in TGF-β1 and methotrexate-induced fibrogenesis, PLoS One 14 (1) (2019), e0208958.
[132] L.M. Norona, D.G. Nguyen, D.A. Gerber, S.C. Presnell, E.L. LeCluyse, Editor's highlight: modeling compound-induced fibrogenesis in vitro using three-dimensional bioprinted human liver tissues, Toxicol. Sci. 154 (2) (2016) 354–367.
[133] P. Godoy, N.J. Hewitt, U. Albrecht, M.E. Andersen, N. Ansari, S. Bhattacharya, et al., Recent advances in 2D and 3D in vitro systems using primary hepatocytes, alternative hepatocyte sources and non-parenchymal liver cells and their use in investigating mechanisms of hepatotoxicity, Cell signaling ADME 87 (8) (2013) 1315–1530.
[134] P.L. Lewis, R.M. Green, R.N. Shah, 3D-printed gelatin scaffolds of differing pore geometry modulate hepatocyte function and gene expression, Acta Biomater. 69 (2018) 63–70.
[135] H. Jeon, K. Kang, S.A. Park, W.D. Kim, S.S. Paik, S.-H. Lee, et al., Generation of Multilayered 3D Structures of HepG2 cells using a Bio-Printing Technique 11, 2017, p. 121 (1).
[136] H. Lee, W. Han, H. Kim, D.-H. Ha, J. Jang, B.S. Kim, et al., Development of Liver Decellularized Extracellular Matrix Bioink for Three-Dimensional Cell Printing-Based Liver Tissue Engineering 18, 2017, pp. 1229–1237 (4).
[137] T. Hiller, J. Berg, L. Elomaa, V. Röhrs, I. Ullah, K. Schaar, et al., Generation of a 3D Liver Model Comprising Human Extracellular Matrix in an Alginate/Gelatin-Based Bioink by Extrusion Bioprinting for Infection and Transduction Studies 19, 2018, p. 3129 (10).
[138] Q. Mao, Y. Wang, Y. Li, S. Juengpanich, W. Li, M. Chen, et al., Fabrication of liver microtissue with liver decellularized extracellular matrix (dECM) bioink by digital light processing (DLP) bioprinting 109, 2020, 110625.
[139] J. Lin, L. Schyschka, R. Mühl-Benninghaus, J. Neumann, L. Hao, N. Nussler, et al., Comparative analysis of phase I and II enzyme activities in 5 hepatic cell lines identifies Huh-7 and HCC-T cells with the highest potential to study drug metabolism 86, 2012, pp. 87–95 (1).
[140] N.A. Kratochwil, C. Meille, S. Fowler, F. Klammers, A. Ekiciler, B. Molitor, et al., Metabolic profiling of human long-term liver models and hepatic clearance predictions from in vitro data using nonlinear mixed-effects modeling 19, 2017, pp. 534–550 (2).
[141] M. Ruoß, G. Damm, M. Vosough, L. Ehret, C. Grom-Baumgarten, M. Petkov, et al., Epigenetic modifications of the liver tumor cell line HepG2 increase their drug metabolic capacity 20, 2019, p. 347 (2).
[142] C. Luckert, C. Schulz, N. Lehmann, M. Thomas, U. Hofmann, S. Hammad, et al., Comparative analysis of 3D culture methods on human HepG2 cells 91, 2017, pp. 393–406 (1).
[143] A. Guillouzo, A. Corlu, C. Aninat, D. Glaise, F. Morel, C. Guguen-Guillouzo, The human hepatoma HepaRG cells: a highly differentiated model for studies of liver metabolism and toxicity of xenobiotics, Chem. Biol. Interact. 168 (1) (2007) 66–73.
[144] R.L. Sison-Young, V.M. Lauschke, E. Johann, E. Alexandre, S. Antherieu, H. Aerts, et al., A multicenter assessment of single-cell models aligned to standard measures of cell health for prediction of acute hepatotoxicity 91, 2017, pp. 1385–1400 (3).
[145] H. Baharvand, S.M. Hashemi, S.K. Ashtiani, A. Farrokhi, Differentiation of human embryonic stem cells into hepatocytes in 2D and 3D culture systems in vitro, Int. J. Dev. Biol. 50 (7) (2004) 645–652.
[146] Z. Heydari, F. Moeinvaziri, T. Agarwal, P. Pooyan, A. Shpichka, T.K. Maiti, et al., Organoids: a novel modality in disease modeling 4, 2021, pp. 689–716 (4).
[147] Z. Heydari, M. Vosough, New platforms for drug screening and toxicology: necessity or need? Modern Medical Lab. J. 2 (1) (2018) 107–109.
[148] K. Kang, Y. Kim, H. Jeon, S.B. Lee, J.S. Kim, S.A. Park, et al., Three-dimensional bioprinting of hepatic structures with directly converted hepatocyte-like cells 24, 2018, pp. 576–583 (7–8).
[149] A. Faulkner-Jones, S. Greenhough, J.A. King, J. Gardner, A. Courtney, W. Shu, Development of a valve-based cell printer for the formation of human embryonic stem cell spheroid aggregates, Biofabrication 5 (1) (2013), 015013.
[150] S. Romanazzo, S. Nemec, I. Roohani, iPSC bioprinting: where are we at? Materials 12 (15) (2019) 2453.
[151] J. Meseguer-Ripolles, S.R. Khetani, J.G. Blanco, M. Iredale, D.C. Hay, Pluripotent stem cell-derived human tissue: platforms to evaluate drug metabolism and safety, AAPS J. 20 (1) (2018) 1–10.
[152] M. Danoy, M.L. Bernier, K. Kimura, S. Poulain, S. Kato, D. Mori, et al., Optimized protocol for the hepatic differentiation of induced pluripotent stem cells in a fluidic microenvironment, Biotechnol. Bioeng. 116 (7) (2019) 1762–1776.
[153] T. Takebe, K. Sekine, M. Enomura, H. Koike, M. Kimura, T. Ogaeri, et al., Vascularized and functional human liver from an iPSC-derived organ bud transplant, Nature 499 (7459) (2013) 481–484.
[154] J.K. Mfopou, M. Geeraerts, R. Dejene, S. Van Langenhoven, A. Aberkane, L.A. Van Grunsven, et al., Efficient definitive endoderm induction from mouse embryonic stem cell adherent cultures: a rapid screening model for differentiation studies, Stem Cell Res. 12 (1) (2014) 166–177.
[155] S. Mathew, M. Jaramillo, X. Zhang, L.A. Zhang, A. Soto-Gutiérrez, I. Banerjee, Analysis of alternative signaling pathways of endoderm induction of human embryonic stem cells identifies context specific differences, BMC Syst. Biol. 6 (1) (2012) 1–13.
[156] D.C. Hay, J. Fletcher, C. Payne, J.D. Terrace, R.C. Gallagher, J. Snoeys, et al., Highly efficient differentiation of hESCs to functional hepatic endoderm requires ActivinA and Wnt3a signaling, Proc. Natl. Acad. Sci. 105 (34) (2008) 12301–12306.
[157] T. Negishi, Y. Nagai, Y. Asaoka, M. Ohno, M. Namae, H. Mitani, et al., Retinoic acid signaling positively regulates liver specification by inducing wnt2bb gene expression in medaka, Hepatology 51 (3) (2010) 1037–1045.
[158] W. Jeong, M.K. Kim, Kang H-W, Effect of detergent type on the performance of liver decellularized extracellular matrix-based bio-inks, J. Tissue Eng. 12 (2021), 2041731421997091.
[159] X. Liu, X. Wang, L. Zhang, L. Sun, H. Wang, H. Zhao, et al., A novel method for generating 3D constructs with branched vascular networks using multi-materials bioprinting and direct surgical anastomosis, bioRxiv (2021).
[160] R. Taymour, D. Kilian, T. Ahlfeld, M. Gelinsky, A. Lode, 3D bioprinting of hepatocytes: core–shell structured co-cultures with fibroblasts for enhanced functionality, Sci. Rep. 11 (1) (2021) 1–18.
[161] F. Xie, L. Sun, Y. Pang, G. Xu, B. Jin, H. Xu, et al., Three-dimensional bio-printing of primary human hepatocellular carcinoma for personalized medicine, Biomaterials 265 (2021), 120416.
[162] Y. Wu, A. Wenger, H. Golzar, X.S. Tang, 3D bioprinting of bicellular liver lobule-mimetic structures via microextrusion of cellulose nanocrystal-incorporated shear-thinning bioink, Sci. Rep. 10 (1) (2020) 1–12.
[163] G.D. Jeffries, S. Xu, T. Lobovkina, V. Kirejev, F. Tusseau, C. Gyllensten, et al., 3D micro-organisation printing of mammalian cells to generate biological tissues, Sci. Rep. 10 (1) (2020) 1–10.
[164] D. Kang, G. Hong, S. An, I. Jang, W.S. Yun, J.H. Shim, et al., Bioprinting of multiscaled hepatic lobules within a highly vascularized construct, Small 16 (13) (2020), 1905505.
[165] S. Mao, J. He, Y. Zhao, T. Liu, F. Xie, H. Yang, et al., Bioprinting of patient-derived in vitro intrahepatic cholangiocarcinoma tumor model: establishment, evaluation and anti-cancer drug testing, Biofabrication 12 (4) (2020), 045014.
[166] C. Yu, X. Ma, W. Zhu, P. Wang, K.L. Miller, J. Stupin, et al., Scanningless and continuous 3D bioprinting of human tissues with decellularized extracellular matrix, Biomaterials 194 (2019) 1–13.
[167] L.M. Norona, D.G. Nguyen, D.A. Gerber, S.C. Presnell, M. Mosedale, P.B. Watkins, Bioprinted liver provides early insight into the role of Kupffer cells in TGF-β1 and methotrexate-induced fibrogenesis, PLoS One 14 (1) (2019), e0208958.
[168] M. Gori, S.M. Giannitelli, M. Torre, P. Mozetic, F. Abbruzzese, M. Trombetta, et al., Biofabrication of hepatic constructs by 3D bioprinting of a cell-laden thermogel: an effective tool to assess drug-induced hepatotoxic response, Adv. Healthc. Mater. 9 (21) (2020), 2001163.
[169] H. Rashidi, N.-T. Luu, S.M. Alwahsh, M. Ginai, S. Alhaque, H. Dong, et al., 3D human liver tissue from pluripotent stem cells displays stable phenotype in vitro and supports compromised liver function in vivo, Arch. Toxicol. 92 (10) (2018) 3117–3129.
[170] T. Hiller, J. Berg, L. Elomaa, V. Röhrs, I. Ullah, K. Schaar, et al., Generation of a 3D liver model comprising human extracellular matrix in an alginate/gelatin-based bioink by extrusion bioprinting for infection and transduction studies, Int. J. Mol. Sci. 19 (10) (2018) 3129.
[171] R. Pimentel, S.K. Ko, C. Caviglia, A. Wolff, J. Emnéus, S.S. Keller, et al., Three-dimensional fabrication of thick and densely populated soft constructs with complex and actively perfused channel network, Acta Biomater. 65 (2018) 174–184.

[172] Y. Kim, K. Kang, S. Yoon, J.S. Kim, S.A. Park, W.D. Kim, et al., Prolongation of liver-specific function for primary hepatocytes maintenance in 3D printed architectures, Organogenesis 14 (1) (2018) 1–12.
[173] T. Grix, A. Ruppelt, A. Thomas, A.-K. Amler, B.P. Noichl, R. Lauster, et al., Bioprinting perfusion-enabled liver equivalents for advanced organ-on-a-chip applications, Genes 9 (4) (2018) 176.
[174] P.L. Lewis, R.M. Green, R.N. Shah, 3D-printed gelatin scaffolds of differing pore geometry modulate hepatocyte function and gene expression, Acta Biomater. 69 (2018) 63–70.
[175] H. Jeon, K. Kang, S.A. Park, W.D. Kim, S.S. Paik, S.-H. Lee, et al., Generation of multilayered 3D structures of HepG2 cells using a bio-printing technique, Gut and liver 11 (1) (2017) 121.
[176] H. Lee, W. Han, H. Kim, D.-H. Ha, J. Jang, B.S. Kim, et al., Development of liver decellularized extracellular matrix bioink for three-dimensional cell printing-based liver tissue engineering, Biomacromolecules 18 (4) (2017) 1229–1237.
[177] K. Kang, Y. Kim, H. Jeon, S.B. Lee, J.S. Kim, S.A. Park, et al., Three-dimensional bioprinting of hepatic structures with directly converted hepatocyte-like cells, Tissue Eng. A 24 (7–8) (2018) 576–583.
[178] S. Massa, M.A. Sakr, J. Seo, P. Bandaru, A. Arneri, S. Bersini, et al., Bioprinted 3D vascularized tissue model for drug toxicity analysis, Biomicrofluidics 11 (4) (2017), 044109.
[179] Y. Wu, Z.Y.W. Lin, A.C. Wenger, K.C. Tam, X.S. Tang, 3D bioprinting of liver-mimetic construct with alginate/cellulose nanocrystal hybrid bioink, Bioprinting 9 (2018) 1–6.
[180] J.-S. Lee, H. Yoon, D. Yoon, G.H. Kim, H.T. Yang, W. Chun, Development of hepatic blocks using human adipose tissue-derived stem cells through three-dimensional cell printing techniques, J. Mater. Chem. B 5 (5) (2017) 1098–1107.
[181] H. Lee, D.-W. Cho, One-step fabrication of an organ-on-a-chip with spatial heterogeneity using a 3D bioprinting technology, Lab Chip 16 (14) (2016) 2618–2625.
[182] S. Knowlton, S. Tasoglu, A bioprinted liver-on-a-chip for drug screening applications, Trends Biotechnol. 34 (9) (2016) 681–682.
[183] J. Zhang, F. Chen, Z. He, Y. Ma, K. Uchiyama, J.-M. Lin, A novel approach for precisely controlled multiple cell patterning in microfluidic chips by inkjet printing and the detection of drug metabolism and diffusion, Analyst 141 (10) (2016) 2940–2947.
[184] N.S. Bhise, V. Manoharan, S. Massa, A. Tamayol, M. Ghaderi, M. Miscuglio, et al., A liver-on-a-chip platform with bioprinted hepatic spheroids, Biofabrication 8 (1) (2016), 014101.
[185] X. Zhou, C. Liu, X. Zhao, X. Wang, A 3D bioprinting liver tumor model for drug screening, World J. Pharm. Pharmaceut. Sci. 5 (4) (2016) 196–213.
[186] A. Faulkner-Jones, C. Fyfe, D.-J. Cornelissen, J. Gardner, J. King, A. Courtney, et al., Bioprinting of human pluripotent stem cells and their directed differentiation into hepatocyte-like cells for the generation of mini-livers in 3D, Biofabrication 7 (4) (2015), 044102.
[187] X. Wang, Y. Huang, C. Liu, A combined rotational mold for manufacturing a functional liver system, J. Bioact. Compat Polym. 30 (4) (2015) 436–451.
[188] L.E. Bertassoni, J.C. Cardoso, V. Manoharan, A.L. Cristino, N.S. Bhise, W. A. Araujo, et al., Direct-write bioprinting of cell-laden methacrylated gelatin hydrogels, Biofabrication 6 (2) (2014), 024105.
[189] Y. Wu, A. Wenger, H. Golzar, X.S. Tang, 3D bioprinting of bicellular liver lobule-mimetic structures via microextrusion of cellulose nanocrystal-incorporated shear-thinning bioink, Sci. Rep. 10 (1) (2020) 1–12.
[190] A. Shpichka, P. Bikmulina, M. Peshkova, Z. Heydari, N. Kosheleva, M. Vosough, et al., Organoids in modelling infectious diseases, Drug Discov. Today 27 (2021) 223–233, https://doi.org/10.1016/j.drudis.2021.08.005. Elsevier.
[191] H.-W. Kang, S.J. Lee, I.K. Ko, C. Kengla, J.J. Yoo, Atala A, A 3D bioprinting system to produce human-scale tissue constructs with structural integrity, Nat. Biotechnol. 34 (3) (2016) 312–319.
[192] L.E. Bertassoni, J.C. Cardoso, V. Manoharan, A.L. Cristino, N.S. Bhise, W. A. Araujo, et al., Direct-write bioprinting of cell-laden methacrylated gelatin hydrogels 6, 2014, 024105 (2).
[193] M.T. Poldervaart, H. Gremmels, K. van Deventer, J.O. Fledderus, F.C. Öner, M. C. Verhaar, et al., Prolonged presence of VEGF promotes vascularization in 3D bioprinted scaffolds with defined architecture 184, 2014, pp. 58–66.
[194] W. Lee, J.C. Debasitis, V.K. Lee, J.-H. Lee, K. Fischer, K. Edminster, et al., Multi-layered culture of human skin fibroblasts and keratinocytes through three-dimensional freeform fabrication 30, 2009, pp. 1587–1595 (8).
[195] C. Norotte, F.S. Marga, L.E. Niklason, G.J.B. Forgacs, Scaffold-free vascular tissue engineering using bioprinting 30, 2009, pp. 5910–5917 (30).
[196] V.K. Lee, A.M. Lanzi, H. Ngo, S.-S. Yoo, P.A. Vincent, G. Dai, et al., Generation of multi-scale vascular network system within 3D hydrogel using 3D bio-printing technology, Cell. Mol. Bioeng. 7 (3) (2014) 460–472.
[197] D. Kang, G. Hong, S. An, I. Jang, W.S. Yun, J.H. Shim, et al., Bioprinting of multiscaled hepatic lobules within a highly vascularized construct 16, 2020, 1905505 (13).