Review article

3D bioprinting of soft materials-based regenerative vascular structures and tissues

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ABSTRACT

Vascularization is a leading limitation for the clinical application of *in vitro* engineered tissue constructs because of the insufficient blood supply in the initial phase after implantation. In spite of decades of progress in the tissue engineering field, vascularization is a major issue that remains unsolved. The advent of 3D bioprinting technology provides a powerful means to resolve the vascularization problem for its advanced time and spatial control, capacity to be changed in size or scale, as well as reproducibility, compared to traditional fabrication processes.

This paper aims to review the recent progress of 3D bioprinting technology in the fabrication of blood vessel, vasculature and vascularized tissue constructs. 3D bioprinting methods and the engineered bioinks for vascular-structure constructions are discussed and compared, followed by a concise discussion of limitations and challenges encountered towards current 3D bioprinting of vascularized tissue. Finally, future research directions on the development of 3D bioprinting processes and bioinks for natural tissue constructions are also discussed.

1. Introduction

Cardiovascular disease (CVD) is the most common cause of death worldwide. An estimated 16.5 million cardiovascular disease (CVD) deaths were reported each year which accounts for 20% of global mortality [1]. While significant progress has made in medical therapy to bring up with potential solutions to reduce the death rate of CVD, cardiovascular transplantation turns out to be the only definitive therapy for CVD for now [2]. Disadvantages exist for allograft transplantation such as paucity of donor tissue, complexities in procurement and handling, transplant rejection and the possibility of disease transmission which have brought difficulties and limitations in CVD therapy [3,4]. Synthetic implants are playing more and more important role in the supply for transplantation. The idea of engineered vessels and vascularized tissues have been presented in tissue engineering for several decades [5,6]. However, engineered tissues are yet to be applied in clinical therapies due to lack of biological functions [7]. One major obstacle regarding biologically functioned tissue constructs is the need of vascular network in engineered tissues [8]. Vascular network is essential in engineered tissues since studies proved that if the engineered tissue thickness is ever to surpass 100–200 μm, vascularized structure must be created for the tissue to transport nutrients and oxygen to tissue cells [8,9]. However, the complexity of blood vessel networks makes the regeneration process complicated. Different types of blood vessels can contain different cell types, range different in sizes from millimeters to micrometers, and support different tissue-specific functions [10,11].

Vascular networks, which are composed of a series of blood vessels, are embedded in most human tissues to serve the function of providing nutrients and oxygen to sustain vitality of the tissues. These tissues supported by the vascular network are referred to as vascularized tissues. When constructing blood vessels or building vascular networks in engineered tissues, several factors have to be considered. Specifically, reproduction of anatomical complex constructs, biomimicry of the extracellular matrix (ECM) [12], recapitulating the diverse biological function [13] are key considerations in blood vessels and vascularized tissue network formation.

In the literature, methods for constructing blood vessels varies depending on whether a single blood vessel or a vascular network

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is created. In terms of constructing single vascular structure, several methodologies have been developed including cell-seeded biodegradable scaffolds, programmed modular cell self-assembly as well as acellular techniques [14]. As for vascularized network, besides the methods used for constructing blood vessels, angiogenic factors and prevascularization are other strategies capable of enhancing vascularization in engineered tissues [15]. All those traditional methods of engineering tissue constructs can be considered as a 2-step procedure: first, mix the cells with growth factors and the scaffold constructs, which are created by natural or synthetic biomaterials; second, immerse the mixture in an *in vitro* bioenvironment [16]. Limitations revealed in this process are low efficiency and inability to control the size of products, which makes it unable to satisfy customized needs [17].

3D bioprinting technology provides a promising way to resolve previous problems for its high efficiency, scalability, time, and spatial control, among other things [18]. It has been applied to medical prostheses [19], organ models [20], and clinical-related tissue constructs for tissue engineering [10,13,20–29]. 3D bioprinting has also made impressive progress in printing two-dimensional (2D) tissues such as skin [13], which has been successfully commercialized. This brings confidence towards printing more complex structures such as blood vessels and vascular networks.

Current significant advances of 3D bioprinting have been achieved by printing hollow geometry as well as the vascularized network geometry [10] with limited biological functions. However, evident gaps exist between synthetic tissues and nature tissues. The main reasons that caused the gaps remained in three fields: bioprinting technique, materials of bioink and fabrication of nature like extracellular matrix (ECM). From the aspect of bioprinting technologies, the resolution of the contemporary printing method still cannot match the precision of real human tissues, such as capillaries (diameter around 10 μm). Considering the bioink, a mixture of materials that is not only easy to print, but also can provides the compatible environment for cell growth need to be developed to support the biological function of the printed structure. For biomimicry of nature like ECM, our understanding of cells and ECM regarding their contents and functions is not thorough. Because of all these reasons, difficulties remain in the construction of highly biological functionalized tissues.

This review aims to provide an overview of the current progress in 3D bioprinting technology in regard to printing blood vessels and vascularized tissue networks. Different types of bioprinting mechanisms such as: micro-extrusion based bioprinting (MEB), drop-let based bioprinting (DBB) and laser-assisted bioprinting (LAB) are reviewed. A variety of bioinks that researchers developed for different printer mechanism using different nozzle and formation process are introduced. The application of blood vessel and vascular network tissue formation are also included. And the current limitations and future research direction in 3D bioprinting of blood vessel and vascular tissue networks are discussed.

2. 3D bioprinting technique

3D printing was first described by Charles W. Hull in 1986. He developed a system for generating 3D objects by creating a cross-sectional pattern of the object via UV light to cure thin layers of a UV curable material and named it 'stereolithography' [30]. Then, 3D printing has been widely studied for its advantages including precise control of the 3D architectures, highly customizable structures, automated and tool-less manufacturing processes, high cost-effectiveness, and others [18]. The success of 3D printing technology then quickly spread to the biomedical field employing special materials such as synthetic polymers, fasteners, and natural cell/

biomaterials (hydrogel, alginate etc.) to form 3D integral constructs. As early as 1999, Odde and Renn [4] used laser direct writing of living cells for the first time. The cells blended with a range of particular materials are guided and deposited on the surface to form 3D patterns on the bottom platform. Recent decades witness the great progress of 3D bioprinting in tissue engineering with the advances in computer science, material science and biomedical engineering [16]. Compared to traditional tissue engineering techniques, 3D bioprinting has the advantage of up to 10–200 μm high-resolution cell deposition [20,31].

Different printing modalities may require different properties for the material and the substrate [32]. They are also based on different printing mechanism which yields different printing procedures and different parameters for blood vessels and vascularized tissue printing, thus, yields different results. Each technique has its strengths, and limitations, but none of the single technique can satisfy all the requirements and concerns needed for fabricating the implantable complex tissue constructs [24,25,28,33,34]. For all 3D bioprinting modalities, the process can be described in the same fashion, (see Fig. 1).

2.1. Micro-extrusion based (MEB) bioprinting technique

The MEB technique is the integration of two processes: extrusion and bioprinting. They are controlled by a fluid-dispensing system and 3D spatial automatic movement controlling system [33]. MEB is one of the most commonly studied methods in 3D bioprinting [10,28,33]. It is the most cost effective printing method among the three above mentioned printing techniques. The working principle of extrusion printing system is that it dispenses the ink using either pneumatic [36] or mechanical (includes piston or screw) dispensers [37,38].

The suitable printing material viscosity for MEB ranges from 30 mPa/s to more than 6×10^7 mPa/s [39]. The materials which meet this requirement include biocompatible hydrogels, copolymer as well as spheroids (a type of cell aggregates) [40]. Materials with higher viscosity is easier to form structure, but come with lower cell viability. Studies reported that the cell survival rates range from 40% to 97% depending on the material and the dispensing system [40]. Some studies used the two-step crosslinking method of the hydrogel to improve cell viability. Specifically, they prepared the partially polymerized alginate-based hydrogel [41]. In MEB, the materials used are non-Newtonian and can be thermally crosslinked. For non-Newtonian material, the increase of shear rate will cause the decrease in viscosity [42], due to pseudoplastic or shear thinning effect. Shear thinning enables the formation of the desired structure by the material when it endures high shear rate at the nozzle head, where the viscosity of the material increase and form the structure after flowing out the nozzle upon deposition [40].

Increasing printing resolution is a challenge to all printing methods. Current MEB technique enables to achieve 5 μm to millimeters wide resolution at low printing speed (5–10 μm/s). But the real tissues requires even nanoscale printing resolution [43] which is still a technology gap for researchers to overcome. Cell viability of MEB can be lower than other printing method, possibly due to the shear stresses applied to the biomaterials while extruding them out from the nozzle. Higher viability can be achieved at the cost of resolution.

Aortic valves, vascular, branched vascular trees have been fabricated using MEB [40]. Adaptation has been made to both the nozzle and printing process which to be more compatible to vascular formation. Yu et al. designed a pressure-assisted freeform fabrication process using coaxial nozzles where two different fluids flow through the tubes, and then meet and trigger the gelation process to print hollow filaments [44] (see Fig. 2 (A)). Later, the
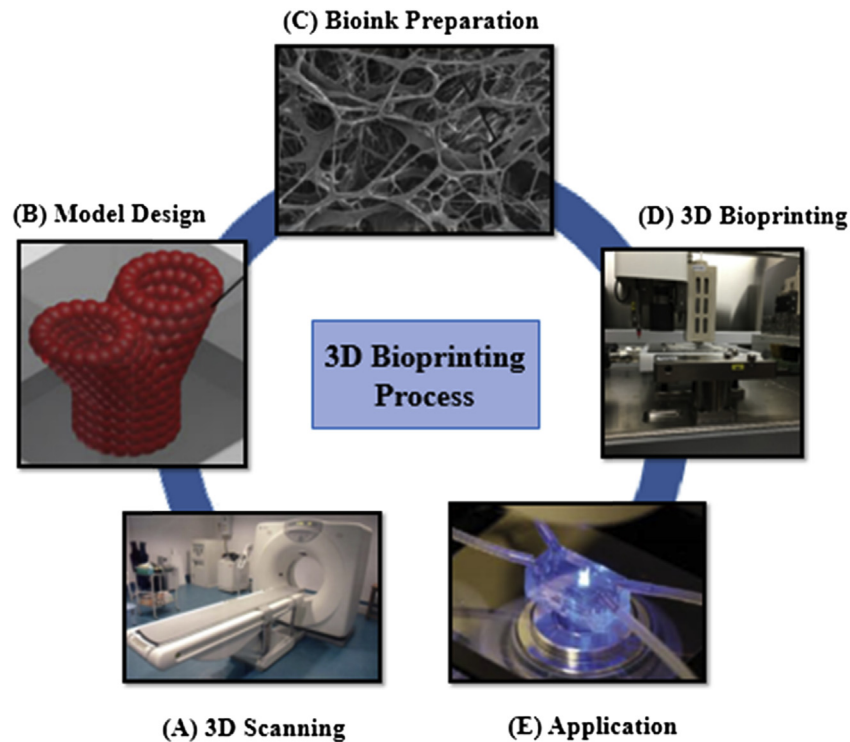
Fig. 1. 3D Bioprinting Process (A) CT scanning/MRI technique gets the 3D image of the damaged part for patients. The 3D images generate the size and geometrical information. The image is adapted from Ref. [13]; (B) CAD models and slicing determine the contour structure and the inner structure while creating G-code. The image is adapted from Ref. [35]; (C) Optimal materials are selected based on constructs' application and the biomaterials are prepared for bioprinting. The image is adapted from Ref. [13]; (D) Bioprinting of the 3D constructs using 3D Bioplotter; (E) Application of 3D bioprinted parts in bioreactor before implantation [13].

printing principle and the mechanics models were established by the same group [45]. Besides, Jia et al. published a method where nozzle size can be tuned to satisfy multiple dimension requirements [36] (see Fig. 2 (B)). They also developed a multi-arm bioprinter that can print multiple materials concurrently with independent motion path and dispensing parameters [46]. Gao et al. [47] modified the printing process by the motorized XY stages and with the coaxial nozzle attached to form hollow filament in precise location. A Z-shaped platform moves up and down to print vertical layers, (see Fig. 2 (C1)). Recently, Gao's group designed a new fabrication method using the rolling process [48], (see Fig. 2 (C2)).

Though MEB is not the first printing technique used in bioprinting, it is the most widely studied one [33] for its advantages including the ability to deposit high cell densities, relatively precise spatial control of the desired constructs compared to other two methods. The flexibility of nozzle size and shape design also facilitate the improvement of MEB in vascular tissue construction. For example, one possible way to form hollow structure and branching structures toward vascular structure is adapting the coaxial nozzles and introducing the rolling process.

2.2. Droplet based bioprinting (DBB) technique

DBB is widely used both in biological and nonbiological printing [40]. DBB yields continuous liquid droplets rather than beads of material to predefined locations. Early versions of DBB printers were modified from commercial 2D inkjet printers. The ink is substituted with bioink, which is composed of biomimetic extracellular matrix and encapsulated cells in the matrix. The droplet is squeezed out of the printhead using thermally induced, piezoelectric, acoustic radiation or electrostatic induced methods [35,40,50]. The main difference between MEB and DBB is the

printing principle. For DBB, the bioink is dispensed out of the nozzle and generates series of droplet under thermal or piezoelectric, acoustic radiation or electrostatic control [51]. Similar to MEB, the bioink used here is composed of biomimetic extracellular matrix and encapsulated cells in the matrix which is biocompatible [38,51].

Multiple hydrogels such as agarose [52], alginate [53], collagen type I [37], Matrigel™ [19] of this type have been studied by researchers. However, the process of squeezing out the droplet from nozzles require materials printed tuned to low viscosity for them to be easier to be dropped. This is one of the major drawbacks using droplet-based printing because it is more difficult to transform low viscosity materials to solid state structure.

Other concerns such as low cell concentration, high risk of exposing cells and materials to thermal and mechanical stress, low droplet directionality, non-uniform droplet size, frequent clogging of the nozzle, and unreliable cell encapsulation also hampers the progress of DBB processes [10,54–59]. However, frequent clogging of the nozzle and unreliable cell encapsulation inhibit the use of these printers in bioprinting field. Practice has been launched to deal with some of the issues. Acoustic-based inkjet printer is developed to print a uniform droplet and avoid the heat and pressure imposed on cells [60]. Later, Tasoglu et al. developed an open-pool nozzle-less ejection system is created to reduce the shear stress at the nozzle tip [61].

2.3. Laser-assisted bioprinting (LAB) technique

LAB uses laser energy to transfer prepared liquid form biomaterials to substrate to fabricate tissue constructs or high-precision patterning of biologics [10,13,32] Typically, LAB is influenced by laser frequency, gas composition, thickness and viscosity
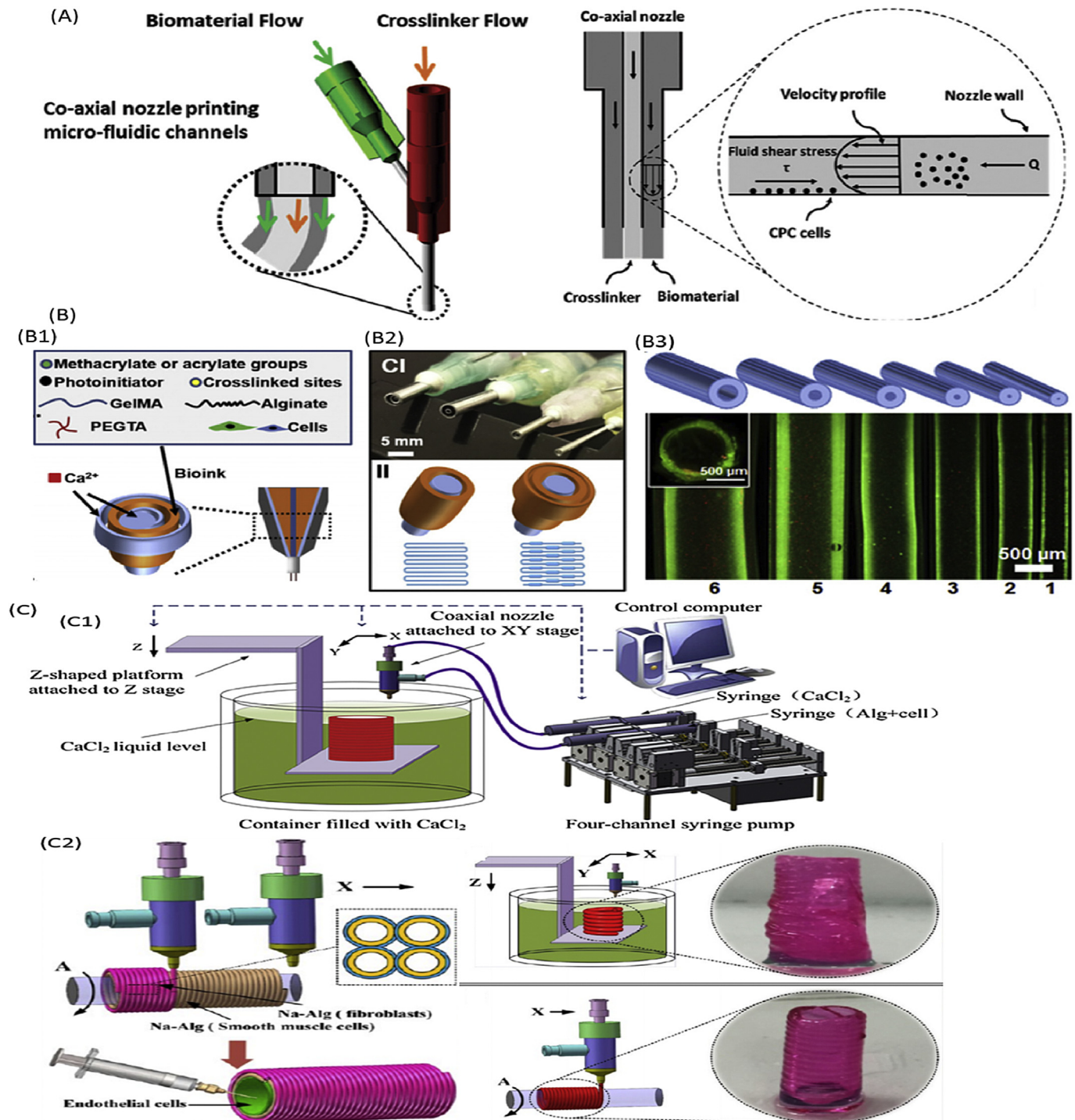
Fig. 2. Coaxial 3D Bioprinting for MEB (A) Coaxial nozzle assembly and associated mechanical forces demonstration [44]; (B) Direct 3D printing perfusable vascular structure [49] (B1) Biomaterial composition and coaxial nozzle demonstration; (B2) The designed multilayered coaxial nozzles and schematic diagram showing fabrication of perfusable hollow tubes with constant diameters and changeable sizes; (B3) Schematic diagram and representative fluorescence micrographs showing the bioprinted perfusable tubes displaying different outer diameters; (C) 3D bioprinting three-layered vascular structure [48] (C1) Fabrication process of 3D alginate vessel-like structures with multiscale fluidic channels; (C2) Vertically printed vascular structure and horizontally printed vascular structure with inner and outer hollow structure.

of the biomaterials, surface tension, wettability of the substrate [13]. A layer of biomaterial is prepared in a liquid solution attached on a support substrate. When laser focuses on the support layer and absorbs the energy, it generates a high-pressure bubble that propels the biomaterial toward the receiving substrate. Compared to the former mentioned printing method, laser-assisted 3D bioprinting has the advantages of precise control to print desired

number of cells [28,62] including even single cell (under 5 Hz frequency, speed up to 1600 mm/s [63]). High cell density achieved by LAB, which is above 10^8 cell/ml is another advantage. Besides, since LAB is nozzle free, clogging problem is eliminated. The viscosity for the material suitable for LAB ranges from 1 mPa/s to 300 mPa/s [32]. Despite the listed advantages, LAB is inefficient both in printing process and material preparation. Low printing speed is

due to rapid gelation kinetics to achieve high shape fidelity [64]. Preparation needs longer time because individual ribbon is required for each printed cells or hydrogels. Accurate cell positioning is difficult to achieve because of the ribbon coating [28,62,63]. Highly costly LAB facility to achieve the printing process is another obstacle [13].

2.4. Comparison of three bioprinting methods

The detailed description of three 3D bioprinting modalities is demonstrated in Fig. 3 (A). The comparison of MEB, DBB and LAB bioprinting methods are summarized in Table 1. The printing parameters of three methods and bioinks for 3D bioprinting
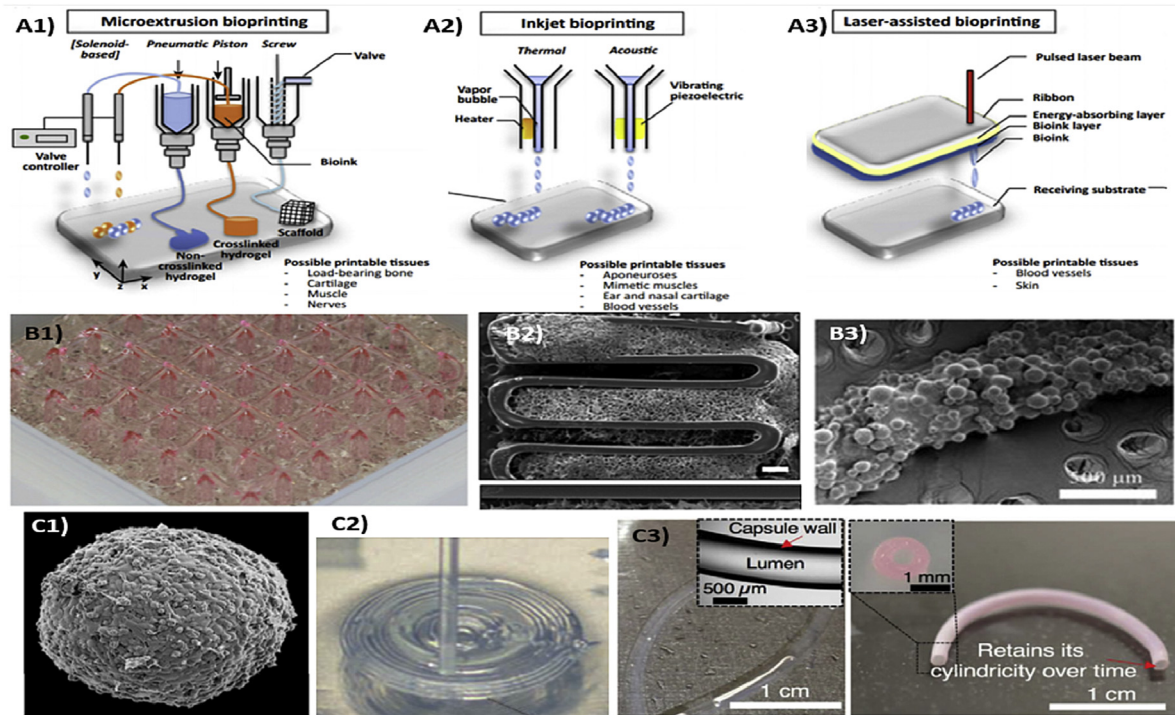

Fig. 3. (A) Bioprinting Methods and Bioinks explored for MEB [10,71] A) Bioprinting Technologies. A1) Micro-extrusion bioprinters use mechanical or pressure working principle; A2) Thermal inkjet bioprinters; A3) Laser-assisted bioprinters use pulsed laser beams focused on an energy-absorbing substrate to generate pressure that propels cell-containing material to deposit onto a receiving substrate. Images adapted from Ref. [71] B,C) Bioink materials. B1) Hydrogel [72]; B2) dECM [73]; B3) Microcarriers [74]; C1) Tissue spheroids [75]; C2) Cell pellet [76]; C3) Tissue strands [77].

Table 1
Comparison of Bioprinting techniques.

	Micro-extrusion Based	Droplet-based	Laser-assisted	Reference
Part I Parameters of 3D Bioprinting Technique				
direct/indirect	Both direct and indirect	Direct	Direct	[28]
Scaffold/Non-scaffold based	Scaffold-based and scaffold-free	Scaffold-based	Scaffold-based	[10]
printing resolution (um)	200	200	Cell size (10)	[31]
Printing Homogeneity	Uniform	Non-uniform	Non-uniform	[10]
3D Bioprinting Capability	High	Medium	Medium	[31]
Degree of Research	High	Medium	Low	[13]
Printing Cost	Low	Medium	High	[31]
Print Speed	Slow (10−50 mm/s)	Fast (1−10,000 droplets per second)	Medium (200−1,600 mm/s)	[13]
Part II Parameters of Bioink				
Cell Density	High (>10^6 cells/ml), Cell-only Bioink	Low (<10^6 cells/ml)	High (10^8 cells/ml)	[13]
Types of Materials for Bioink	Cell-free, Cell-laden Cell-only Bioink	Cell-free, Cell-laden Bioink	Cell-laden Bioink	[28]
Vertical mechanical structure quality	High	Poor	Medium	[54]
bioink viscosity (mPa/s)	High (30 - >$6*10^7$)	Low (3.5−12)	Medium (1−300)	[13]
Part III Advantages and disadvantages of Bioprinting Methods				
Advantages	High cell density, Spatial control, Support scaffold-free printing	High Speed, Medium resolution	No clogging issue	[10,13,17,28,31, 33,45,65−70]
Disadvantages	Moderate speed, Clogging issue (at 150 μm)	Low cell concentration, Non-uniform droplet size, Clogging	Difficult to target precise cell position, High cost, Low cell viability, Un-uniform size of ink droplet	

techniques are listed in the table. The advantages and disadvantages for each modality are demonstrated as well.

3. Bioinks for vascular and vascularized tissue

Above discussed bioprinting methods provide a variety of approaches for soft tissue and scaffold fabrication. The functionality of printed constructs depends on both the printing method (printing mechanism, resolution etc.) as well as the chosen biomaterials and cells used for printing. Moreover, the bioinks designed for each printing method varies based on their bioprinting mechanism and the printing parameters. Likewise, for each printing method, when printing constructs with different function or geometry, the features of designed bioinks varies. This variety is the result of not only manufacturing ability, but also many other features of the ideal construct including mechanical integrity, stability, insolubility in cell culture, biodegradability, biocompatibility, toxicity and cell adhesion etc.

Among the bioinks used in MEB bioprinting methods, Bioink materials can be categorized into two major types depending on their printing processes [32]. One type is scaffold-based bioink materials where the printed constructs need a scaffold support. Another type is scaffold-free bioink materials where cells are printed without the use of an exogenous support [32]. For each type of bioinks, researchers have fabricated blood vessels in a different scale and structures, vascular networks as well as the thick vascularized tissue *in vitro* [28,32,72,78].

3.1. Scaffold-based bioinks

The scaffold-based bioprinting is the most common bioprinting technique for tissue constructions. Scaffold plays an important role in tissue regeneration where they provide a space for cell adhesion [79] as well as biological cues for cell differentiation [80]. Typically, scaffolds with a high porosity yields large surface area enabling cell adhesion and formation of blood vessels. Previously studied scaffold-based bioinks can be generally classified into three categories: hydrogel, decellularized extracellular matrix (dECM) and microcarrier.

3.1.1. Hydrogel
Hydrogel is composed of a series of hydrophilic polymer chains that are crosslinked through physical bonding such as thermal, chemical bonding like $CaCl_2$ solution [41,81], or enzymatic bonding, in the presence of water [82]. Hydrogels can be categorized into natural hydrogels which include gelatin [83], fibrin [84], alginate [44], chitosan [45] and hyaluronic acid [85], and synthetic hydrogels such as Poly(2-hydroxethyl methacrylate) (PHEMA) [86], Poly(vinyl alcohol) (PVA) [86] and Poly(ethylene glycol) (PEG) [38]. In order to mimic the *in vivo* environment, properties of hydrogels such as rheology (viscosity, viscoelasticity, shear thinning, yield stress), crosslinking mechanism, physical behavior, and solute transportation are key to human tissue regeneration [86]. The choice of hydrogel in bioinks is based on both the properties and the requirements of the printing method. Though hydrogels are widely studied and accepted by researchers for its biocompatibility, printability and tunability in bioprinting, the weak mechanical strength, as well as the shape fidelity hinder their further applications [87]. To deal with these issues, researchers have fabricated hydrogels with higher toughness and succeeded 3D bioprinting the tough hydrogel [88]. Incorporating hard and biodegradable synthetic non-toxic polymers to enhance mechanical properties, provided a better shape fidelity in the meantime [89]. Another way to achieve high mechanical structure is incorporating carbon nanotubes (CNTs) in printing biomaterials [90–92]. Dolati et al. [90]

direct printed vascular conduits where conduits were reinforced with CNTs which turns out to improve the mechanical properties and bioprintability as well. They also concluded that for large-scale tissue fabrication, CNTs could be replaced with natural protein nanofibers.

A great number of researchers are working on the fabrication of vascular and vascular network involving different types of hydrogels [93,94]. Alginate and GelMA are the two commonly studied hydrogels for their good compatibility compared to other synthetic ones. Jia et al. [95] tuned the cell-laden alginate bioink and found that consistent cell distribution and high cell viability can be achieved by homogeneous cell suspension while using extrusion bioprinters. Yu et al. used the coaxial nozzle to print alginate to mimic the natural vascular system [44]. The same group used chitosan powder which is another natural material used in vascular formation [45]. However, Ozbolat et al. [44] reported that compared to chitosan, alginate based structure exhibit higher mechanical strength.

Methacrylated gelatin (GelMA) is another widely used biocompatible material in printing vascular structures. GelMA is denatured from collagen and conjugated amine to its side groups. Bertassoni et al. [96] printed cell-laden photolabile GelMA via direct-write printing with varying range of concentrations, cell densities and mechanical properties. A series of experiments revealed that higher mechanical strength facilitates the printing of pre-polymerized GelMA. Later, GelMA is combined with other synthetic polymers such as polyethylene glycol diacrylate (PEGDA) and photo-initiator into composite materials [97]. Jia et al. [49] designed a more complicated bioink consisting of GelMA, sodium alginate, and poly (ethylene glycol)-tetra-acrylate (PEGTA). A multilayered coaxial MEB is used to direct print 3D vascular structure. The bioink is ionically crosslinked by calcium ions and stable constructs are formed by photocrosslinking GelMA and PEGTA. Rutz et al. proposed a general method of fabricating multimaterial bioinks for 3D printing tunable, cell-compatible PEG [98]. Precursor solution with lightly crosslinked, soft hydrogels is prepared ahead of printing. These bioinks show the prospects to print various tissue constructs including vascular structure. Recently, Ouyang et al. [99] discovered a non-viscous photo-crosslinkable hydrogels for in situ crosslinking. This method excels in maintaining high cell viability as well as tunable cell behavior. Heterogeneous and hollow filaments are printed using this method which brings hope of printing blood vessels and vascular network tissues.

3.1.2. Decellularized extracellular matrix (dECM)
Extracellular Matrix (ECM) serves as a medium that foster cell attachment, proliferation, signaling, and tissue development. ECM is composed of cell-secreted molecules. dECM has been developed to recapitulate natural tissue environment [32]. dECM material is by removing the original cells from the tissue using chemical, physical and enzymatic processes, but leaving the ECM components. DNA quantification assays are launched to determine the degree of decellularization [73]. To form a bioink using dECM, the material is solubilized in a gel-like form. dECM can provide the complex natural tissue environment which are able to reconstitute the intrinsic cellular morphologies and functions. This is a property that the majority of the matrix materials used so far for bioprinting cannot achieve [73]. Pati et al. showed that there are no cytotoxicity of printed cell laden dECM/Polycaprolactone (PCL) constructs [73]. PCL acts as the supporting material for maintaining the stability of the constructs. However, dECMs are difficult to acquire because the source comes from tissues of human body. Bioprinted tissues using dECMs also has a risk of causing immunoreaction by the recipients [38].

3.1.3. Microcarrier

Biomaterials incorporating microcarriers compound system is another kind of bioink developed for 3D bioprinting. Microcarriers act as a reinforcement block in building constructs with large surface area to transfer cells. Common materials for microcarriers are dextran, plastic, glass, gelatin and collagen [38]. One notable advantage using microcarriers is the high cell density with high cell viability in bioinks. Besides, microcarriers can support the differentiation of stem cells to desired lineages [38]. Levato et al. used microcarriers in hydrogel to form the 3D constructs [85]. Levato's study showed that cells that are integrated with microcarriers merely suspend in hydrogels, which improves interaction, and aggregation of cells as well as stem cell differentiation. However, degradation of the microcarrier material can be toxic to cells, and using microcarriers can cause clogging of the nozzle tip. There are not as many researches launched in microcarriers, but research regarding microcarriers is highly meaningful to improve the cell density as well as not losing resolution in MEB.

3.2. Scaffold-free bioinks

Scaffold free bioinks are cell-adhesive, structurally supportive bioinks for vascular and vascular network fabrication. Scaffold-free bioinks can be extruded from the nozzle to form the complex vascular structure which then followed by cellular integration or cell suspension perfusion. There are three types of scaffold-free bioinks for MEB bioprinting: tissue spheroids [100], cell pellet and tissue strands [38], (see Fig. 3 (C)). Compared to scaffold based bioinks, the process of fabricating scaffold free bioinks are more complicated and difficult. Thus, relatively less research are reported for scaffold-free bioinks.

3.2.1. Tissue spheroids

Tissue spheroids are cell aggregates in 200–400 μm diameter which serve as building blocks in regenerative medicine. The spheroids are formed either by using a micro-well where cells are cultivated or using gravity to aggregate the cells and drop them [32]. After the spheroids are formed, they are assembled into a dispensing tip to be printed one by one. A successive of spheroids are dispensed on the bottom substrate and the printed spheroids fused together then formed larger scale patterned tissues.

3.2.2. Cell pellets

A cell pellet is a concentration of cells that are formed at the bottom of the conical tubes through gravitational force [32]. Forming cell pellets does not need complex process, but cell viability is relatively low because of insufficient oxygen supplier during the preparation [32]. This type of bioink has been utilized to fabricate aortic constructs [76] and nerve grafts [101].

3.2.3. Tissue strands

Tissue strands are a promising way of building large scale tissue constructs with high cell density as well as preserving high cell viability [31]. The hollow and porous alginate tubes are formed before inserting cells into the tube. Cells are injected and packed into hollow alginate tubes with high density [32]. The porous structure provides enough oxygen and nutrients to the cells to achieve higher cell viability. This method has been used in printing cartilage [70].

4. Application

4.1. Blood vessels

With the development of printing techniques and the material fabrication, a growing number of biomaterials can be printed to reliable constructs with biological functions suitable for implantation. Based on the scaffold and scaffold-free construction principles, a number of works have reported successful printing of vascular-like structures [102–104] (see Fig. 2).

4.1.1. Scaffold based bioprinting

Previous studies regarding scaffold based tissue engineering have shown that scaffolds act as a supporting structure which provide a place for cells to adhere and proliferate [105]. Different structure, different shape and different printing materials will provide different biological cues for cell proliferation and differentiation [32]. Skardal et al. [106] used cell-free thiol-modified hyaluronic acid, gelatin, and gold nanoparticles as dynamic, multivalent crosslinkers to form the tubular constructs that support cell proliferation and matrix remodeling. Later, Bertassoni et al. [96] used cell-laden GelMA hydrogels to form the vascular construct. Both methods print hydrogel based material as scaffolds to form vascular structure using layer by layer method. Another groups of researchers [44,45,47] invented coaxial nozzle to extrude alginate-based hydrogel and crosslinking material (such as $CaCl_2$) separately to form the hollow structure. Zhang's experiment result showed a printed blood vessel with roughly 100 μm wall thickness, which is thin enough for oxygen and nutrition transition [42]. The advantage of the coaxial printing system is that it can directly form the hollow structure while printing. Nevertheless, this technique still faces shortcomings such as limited cell viability, limited tissue versatility and undesired mechanical properties of print vessel [42]. Later, a microfluidic print head was developed allowing the printing of low viscosity materials and multiple materials in various dimensions, as shown in Fig. 3 (B) [49]. A branched vascular structure was formed using a horizontal-direction printing with a rod recently by Gao et al. [107].

4.1.2. Scaffold free bioprinting

The scaffold-free method was developed for printing vascular-like structure, one typical material form used is spheroids [10]. MEB is more adaptable for scaffold-free bioink (or cell-only bioink) [33]. A layer-by-layer deposition in the form of spheroids as building blocks is firstly proposed by Mironov et al. [87]. Horizontal printing is another way to form a vascular shape using pre-fabricated tissue strands [75]. Using Organovo [108], a commercial 3D bioprinter, researchers printed cylindrical agarose strips (support material) and cellular bioink alternatively to form designed vascular construct in horizontal position. This eliminated the need of spheroids, but the lengthy printing process made it hard for the cells to survive through the whole process. More reliable printing method has been developed later by introducing a concentric mold in printing process: Tan et al. [108] built a blood vessel vertically using alginate-based spheroids and direct molding technique. 3D hydrogel mold was fabricated by depositing alginate microdroplet on calcium substrates for gelation. Vascular tissue was then formed via fusion of the spheroids which consist of endothelial cells (ECs) and smooth muscle cells (SMCs) by robotically placing them into the molds mentioned above using a 3D printer. Despite the progresses made in scaffold-free printing, laborious material preparation process and difficulties in printing operation, biocompatibility, time of printing and scale up problem are main concerns that prevent further the development of the above mentioned techniques.

4.2. Vascularized tissue network

In the past decades, vascularization played as the key limitation in the fabrication of complex thick tissue and functional organs. 3D

bioprinting is a promising technique to create desired vascular network pattern. Current researches can be categorized into microscale (which has a diameter < 100 μm) and macroscale (which has a diameter > 100 μm) vascular network generation. Progress is significant in both microscale and microscale vascular network fabrication (see Fig. 4).

Several methods have been adapted to generate the clinically-relevant volume of tissues with vascular structure [23,28,72,78,109–112]. One typical method is to use sacrificial material, such as Pluronic F127 [112], agarose [109], carbohydrate glass [110]and gelatin [113]when printing the vascular hollow structure. Sacrificial materials are temperature sensitive [10,79,86]. For Pluronic F127, it can transform from solid state to liquid when temperature cool down below ca. 4 °C [72], (detailed process see Fig. 4 (A)). GelMA is the type of material with opposite property of Pluronic F127. For GelMA, gelation happens below approximately 23 °C. By lowering temperature, GelMA crosslinks and forms synthetic ECM matrix while Pluronic F127 liquifies and flow out, leaving the hollow network structure. Kolesky et al. [72,78] also tuned concentration, and degree of methacrylation of GelMA in bioinks to modify the shear yield stress and elasticity of the aqueous GelMA systems. Moderate cell concentration (2×10^6 cell/ml fibroblast cells) in the 15 wt/v% GelMA ink were printed and cell viability as high as 82% were maintained after 7 days of printing, (detailed process see Fig. 4 (B)). Bertassoni et al. [109] use the same methodology to form vascular network in synthetic tissue substituting Pluronic F127 by agarose as sacrificial material. Interestingly, agarose fibers did not adhere to the surrounding GelMA hydrogels, thus it can be easily removed via aspirating with the

light vacuum or manually pulling out. Then, the vascular network was formed in the hydrogel tissue. Incorporating the sacrificial material in tissue fabrication process helps to lower the requirement for printing technique and material property, but may also complicate the printing process. Miller et al. [110] used the similar process to fabricate vascular network using carbohydrate glass as sacrificial material, (detailed process see Fig. 4 (C)).

Direct printing is another method to form vascular network [96,114]. Bertassoni et al. [96] direct-printed the photolabile cell-laden methacrylated gelatin (GelMA) hydrogels using a metallic piston. Kang et al. [114] used integrated tissue−organ printer (ITOP) to fabricate stable, human-scale tissue constructs of any shape. They incorporated vascular network into the printed tissue constructs which facilitates diffusion of nutrients to printed cells, thereby overcoming the diffusion limit of 100−200 μm for cell survival in engineered tissues. The coaxial nozzle which can directly form hollow structure are also utilized to form vascular network [44,45,47,48].

The vascular system in thick tissue and human organ *in vivo* is characterized by a multi-scale organization, which is not practical by most bioprinting technologies [115]. The integration of direct and sacrificial printing methods provided a way to fabricate multi-scale network. Lee et al. [116] proposed this idea. They 3D bioprinted larger (lumen size of ~1 mm) fluidic vascular channels and connected the channels with capillary network formed through angiogenic sprouting from the edge of large channels. Two large fluidic vascular channels are formed through sacrificial printing method, and the capillary is formed in preprinted fibrin cell mixture. After perfusion of vascular channels, cells migrated to the
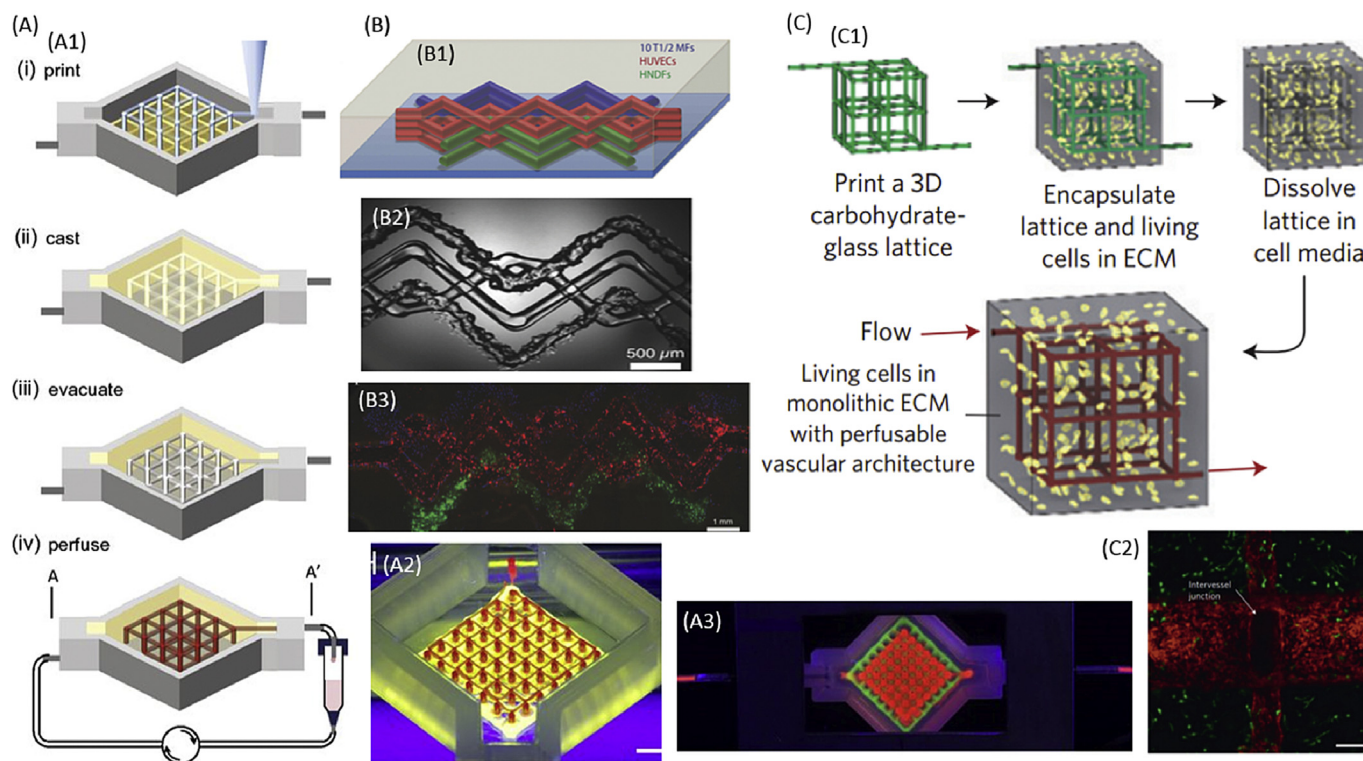

Fig. 4. Three-dimensional vascularized tissue fabrication. (A1) Schematic illustration of the tissue manufacturing process [72] (i) Fugitive (vascular) ink, which contains and thrombin, and cell-laden inks, which contain gelatin, fibrinogen, and cells, are printed within a 3D perfusion chip; (ii) ECM material, which contains gelatin, fibrinogen, cells, thrombin, and TG, is then cast over the printed inks; After casting, thrombin induces fibrinogen cleavage and rapid polymerization into fibrin in both the cast matrix, and through diffusion, in the printed cell ink. Similarly, TG diffuses from the molten casting matrix and slowly cross-links the gelatin and fibrin; (iii) Upon cooling, the fugitive ink liquefies and is evacuated, leaving behind a pervasive vascular network, which is (iv) endothelialized and perfused via an external pump; (A2) Interpenetrated sacrificial (red) and cell inks (green) as printed on chip (Scale bar: 2 mm); (A3) Printed tissue construct housed within a perfusion chamber; (B) 3D bioprinted vascular-like structures with GelMA and Pluronic F127 bioinks [78]; (C) 3D bioprinting vascularized cubic tissue constructs [110]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

edge of fluidic channels and gradually formed the capillary network. This result provides a feasible solution to connect capillary network with large channels and exhibit the potential in printing desired 3D vascular patterns in thick tissues. In the same year, they also developed a 3D bioprinting method to create a vascular channel with perfused open lumen using only cells and biological matrices, (see Fig. 5). Capillary network was observed on the vessel surface, which can be explained as active angiogenic sprouting as well [113].

Some researchers are exploring direct printing microvascular network in thick tissue. In 2010, Wu et al. [42] omnidirectionally printed a 3D microvascular network within a hydrogel reservoir using the angiogenesis characteristics of living tissue. Maturation of the printed structure is a challenge for current printing technology. Recently, Lee et al. [117] fabricated a capillary-like network in the printed liver tissue using a multi-head building system. The bioink was infused into the PCL to induce the formation of capillary-like networks and hepatic tissue growth. The study proved the importance of capillarization that it can greatly improve the cell viability as well as protein secretion, and showed the potential of vascularization for printed tissue with complex structure as well.

Though a number of works have been done, 3D printing of macro-micro integrated vascular network is yet to be achieved because of the current technology limitations in time and spatial resolution for printing capillary network (~10 μmin diameter) at single cell level. More researches are needed to help create the complex structure in a more organized and controllable way by the advancement of 3D bioprinting technology. Angiogenesis is a potential way to partially solve this issue, but higher resolution printer is also highly demanded.

5. Future directions

In tissue engineering, one of the most important challenges that researchers must surpass is vascularization. Studies have proved that if the engineered tissue thickness is ever to surpass 100–200 μm, vascularized structure must be created for the tissue to provide nutrients and oxygen to tissue cells [8,9]. 3D Bioprinting is a promising solution to solve this issue. 3D bioprinting has a powerful strength to replicate micro-architecture with precise time and spatial control.

The challenge of vascularization can be further specified as fabricating functional vascular tree structure including macroscale and microscale vessels, differentiating the inserted stem cells to the desired lineage, and aggregating to act as integral functional constructs and maintained adequate mechanical strength [13]. Current research is still struggling with the first task, leaving a big knowledge gap between fabricated tissue and real functional tissue.

In order to close the research gap and finally fabricate functional tissue, one important challenge for researchers is scale up of printed tissue. More specifically, most of the current research can only print a small piece of tissue or a piece of the vascular structure instead of a human-scale tissue or vascular network. One reason behind this is the speed of current 3D printing methods is not fast enough to print a whole vascularized structure to immediately satisfy a real demand. Kolesky et al. [35]. estimated that to print an adult human liver using a single nozzle of 200 μm diameter, it would take 3 days. Rapid 3D printing design is strongly needed that can print complex vascular structure in any fashion needed.

Due to the complex structure of the vascular system, a complete biomimicry of the blood vessels with exact shape and function is barely possible, not to mention millions of capillaries in complex tissues. Macrovascular structures have been created by many researchers, while few managed to build microvascular system, partly because of the limitation of resolution of contemporary 3D bioprinting technologies. More research need to be done to eventually create a microvascular network in tandem with the rest of macro-sized tissue.

Another challenge regarding 3D bioprinting is to adapt technologies designed to print molten plastics and metals to print sensitive biomaterials [13]. This difficulty is caused by the complexities of biological issues, for example, choice of biomaterials with desired functions, cell types, growth factors and technical challenges related to the sensitivities of living cells and the construction of tissues. Specifically, there are two central problems to consider. The first is to reproduce the complex micro-architecture
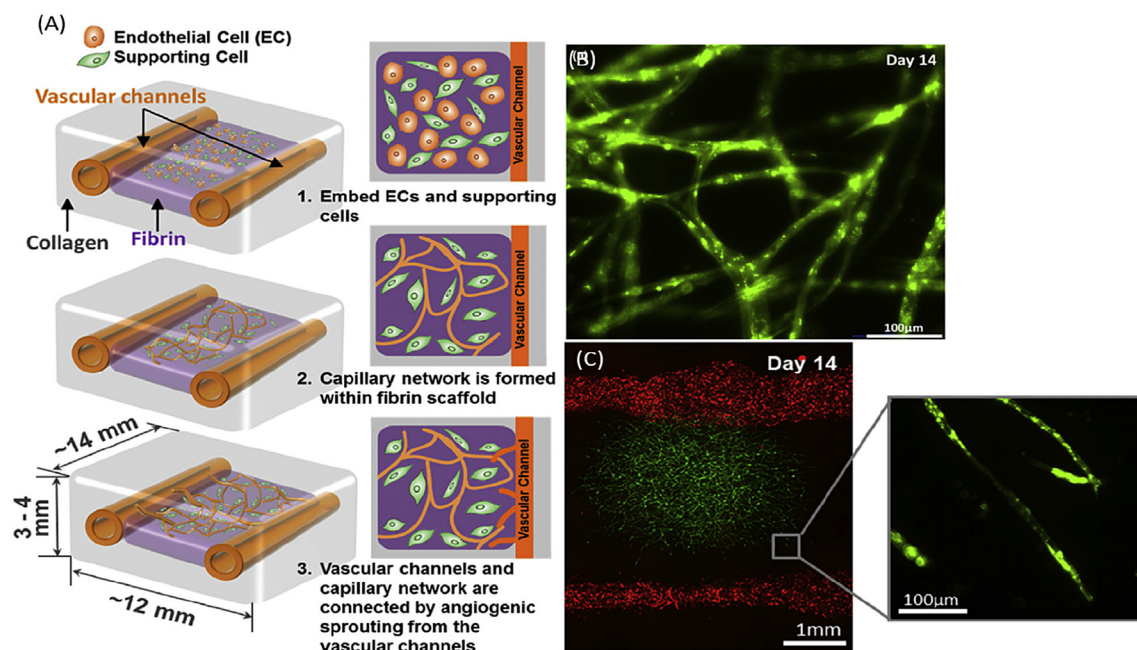

Fig. 5. Cell-secreted Collagen I can improve cell-cell adhesion, tissue formation and maturation [116].

Table 2
Integrated challenge for 3D bioprinting vascular and vasculature.

	Challenge for Bioprinting	Challenge for Bioink	Reference
Microextrusion-based	Trade-off among cell viability, Printing speed, Nozzle diameter (resolution)	Shear thinning material only, Shear rate cause cell death in higher bioink viscosity	[13,27]
Droplet-based	Low cell concentration, Risk of exposing cells and materials to thermal and mechanical stress, Low droplet directionality, Nonuniform droplet size, Frequent clogging of the nozzle, Unreliable cell encapsulation.	Lower viscosity bioink, Lower cell density	[10,51]
Laser-assisted	Lowest cell viability among three methods, Difficult to target precise cell position, High cost.	Limited materials which fuse but do not decompose under the laser beam	[10,13]
Common issue for all bioprinting methods	Uniform Printing Modality	Un-uniform printing modality need different bioink properties	[33,68,98]
	Scale up	Bioink with high similarity to natural ECM	
	Fabricating the tissue with exact same functionality	Fabricating the tissue with exact same functionality	
	Vascularization, current research is limited to preliminary state	Vascularization, current research is limited to preliminary state	

of ECM components. The second is to print multiple cell types in sufficient resolution to achieve multiple biological functions.

Since the appearance of 3D Bioprinting technology, great progress has been made. Still, many limitations regarding 3D Bioprinting of blood vessels and vascularized tissues exist which are listed above (Table 2). Possible solutions require more advanced technique, material modification and further understanding of biological structure and biological performance.

Speaking of 3D bioprinting technique, more advanced printing strategies are highly desired. The optimal 3D printing method is to be able to print both the large-scale blood vessels as well as the small-scale capillaries at the same time which can eliminate the problem of vessel connection when it comes to complicated branched structure. Time-wise efficient is also a requirement for ideal 3D bioprinting. The printing speed will also influence the printed structures' performance and the printing resolution. However, lengthy printing process will impact cell viability both for the cells remained in the printing cartridge and the cells contained in the printed constructs as well [118]. In this case, reducing time without decreasing printing resolution and the printed constructs' performance is an ideal way for 3D bioprinting. Current printing speed for viscous biomaterials (especially when they contain cells) in EBB is around 10 mm/s to 50 mm/s. Previous study has shown that it would take 3 days to print an engineered tissue construct with a volume of ca. 1000 cm^3, comparable to a typical adult human liver, using a single (200 μm) nozzle at typical printing speeds [35]. However, the same volume could be printed in 1 h using a 64 multi-nozzle array. Furthermore, incorporating multiple nozzles in the 3D printing system to print at the same time also enables the printing of more complicated structure. This can further realize the bio-mimicry the biological structure of vascularized tissue. Rolling is another possible solution to the scale-up issue. Bioprinting blood vessel structure, is not easy to achieve using a single roller. For larger scale and regular repetition pattern, roll to roll system has been implemented in many another field including flexible electronics [39]. Introducing this technique in 3D bio-fabrication of blood vessel and vasculature tissue is a promising solution for current limitations.

As mentioned in the previous section, there are many biomaterials fabricated for various types of 3D bioprinter, such as hydrogel, dECM and microcarriers. Hydrogels have been widely studied including synthetic ones and natural ones. But there are multiple strict requirements regarding 3D printing bioink, such as shear thinning property, the property of viscosity of a Non-Newtonian fluid depending on time, temperature and shear rate, and thixotropic property when considering MEB printing modality. Only limited number of biomaterials have the shear thinning and

thixotropic property which is a disadvantage for extruding the material out of the nozzle, let alone when considering cell compatibility of the material. Material compatibility becomes a concern when cells are added for printing. Fabricating new biomaterial or modification of the current biomaterial is highly needed: one type of material is difficult or unable to satisfy these requirements, so one of the possible solutions is to combine different kinds of material (composite material) that can exhibit multi-properties. The materials chosen as the ingredients are important because the compatibility and final performance highly depend on the composition of the materials. Nano-structured materials have unique property, so incorporating nanomaterial such as nanoparticles in the bioink is a way to modify the mechanical properties of the bioink. More unknown properties and effects of nanomaterial incorporation need to be explored by researchers.

Cell performance will influence the biological function of the printed constructs which is always a complicated issue to study. Cell viability is one of the essential factors to the printed tissue performance. In the literature, cell viability is usually studied for two weeks or less, which is far from enough for us to fully understand the cell behavior and construct performance. Longer studies need to be done regarding this matter. Stem cell has a strong differential potential which can be further studied in 3D bioprinting field. The majority of works towards bioprinting vascular or vascularized tissue networks used highly specialized cells such ECs, SMCs, Human Umbilical Vein Endothelial Cells (HUVECs) etc. Though the use of specialized cells has the advantage of phenotypically relevance, physiological functionality, isolation property and easiness to use, it can lead to poor *in vitro* differentiation and limited expansion capacity. So, the use of stem cells (especially Human umbilical vein endothelial cell) for vascular and other soft tissue formation is highly promising. Stem cells are pluripotent cells and are able to differentiate into other cell types upon exposure to correct physical and chemical guidance cues [90]. Stem cells, though studied for a long time in tissue engineering, have not been popular in 3D bioprinting area for its difficulties in operation.

As a type of tissue engineering method, 3D bioprinting has the unique advantages in specific dimensional control (cell seeding and geometric control), complex structure fabrication and so on, which makes it a promising method to achieve tissue regeneration and push forward the development of tissue engineering.

6. Conclusion

This paper reviews 3D bioprinting techniques and bioinks used for vascular-structure constructions. Among the three types of 3D

bioprinting methods: MEB, DBB and LAB, MEB is the most widely studied method for vascular-structure printing and huge progress has been made in printing thick vascularized tissue constructs using MEB. Future efforts need to be put in the evolution of modified or improved printing processes as well as the discovery of advanced bioinks for manufacturing functional vascularized tissue constructs. Current knowledge of tissue construction is still far from fabricating tissues with structure and biological functions like real one due to the limitation of printing scale and resolution, as well as the properties of bioinks, such as, mechanical properties, biological cues for cell-cell interactions and propagations. Some potential directions of research are suggested regarding these limitations. With the advent of bioprinting processes and the various compatible engineered bioinks for bioprinting, 3D bioprinting vascularized tissue constructs has a promising future in resolving the vascularization issue in tissue regeneration field. 3D bioprinting technology will be a key technology in tissue engineering.

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References

[1] Mozaffarian. Heart Disease and Stroke Statistics-2015 update: a report from the American Heart Association (vol. 131, pg e29, 2015). Circulation 2016;133(8). E417-E.
[2] Garbern JC, Lee RT. Cardiac stem cell therapy and the promise of heart regeneration. Cell Stem Cell 2013;12(6):689—98.
[3] Scott JM, Armenian S, Giralt S, Moslehi J, Wang T, Jones LW. Cardiovascular disease following hematopoietic stem cell transplantation: pathogenesis, detection, and the cardioprotective role of aerobic training. Crit Rev Oncology/Hematology 2016;98:222—34.
[4] Odde DJ, Renn MJ. Laser-guided direct writing of living cells. Biotechnol Bioeng 2000;67(3):312—8.
[5] Griffith LG, Naughton G. Tissue engineering—current challenges and expanding opportunities. Science 2002;295(5557):1009—14.
[6] Khademhosseini A, Langer R. A decade of progress in tissue engineering. Nat Protoc 2016;11(10):1775—81.
[7] Jain RK, Au P, Tam J, Duda DG, Fukumura D. Engineering vascularized tissue. Nat Biotechnol 2005;23(7):821—4.
[8] Mironov V, Trusk T, Kasyanov V, Little S, Swaja R, Markwald R. Biofabrication: a 21st century manufacturing paradigm. Biofabrication 2009;1(2):022001.
[9] Shaw CJ, ter Haar GR, Rivens IH, Giussani DA, Lees CC. Pathophysiological mechanisms of high-intensity focused ultrasound-mediated vascular occlusion and relevance to non-invasive fetal surgery. J R Soc Interface 2014;11(95):20140029.
[10] Datta P, Ayan B, Ozbolat IT. Bioprinting for vascular and vascularized tissue biofabrication. Acta Biomater, 15, 1—20.
[11] Reece JB. Campbell biology/Jane B. Reece ... [et al.]. Boston: Benjamin Cummings; 2011. imprint of Pearson.
[12] Michel G, Tonon T, Scornet D, Cock JM, Kloareg B. The cell wall polysaccharide metabolism of the brown alga Ectocarpus siliculosus. Insights into the evolution of extracellular matrix polysaccharides in Eukaryotes. New Phytol 2010;188(1):82—97.
[13] Murphy SV, Atala A. 3D bioprinting of tissues and organs. Nat Biotech 2014;32(8):773—85.
[14] Nerem RM, Seliktar D. Vascular tissue engineering. Annu Rev Biomed Eng 2001;3(1):225—43.
[15] Rouwkema J, Rivron NC, van Blitterswijk CA. Vascularization in tissue engineering. Trends Biotechnol 2008;26(8):434—41.
[16] Demirci U, Montesano G. Single cell epitaxy by acoustic picolitre droplets. Lab a chip 2007;7(9):1139—45.
[17] Novosel EC, Kleinhans C, Kluger PJ. Vascularization is the key challenge in tissue engineering. Adv drug Deliv Rev 2011;63(4):300—11.
[18] Rengier F, Mehndiratta A, von Tengg-Kobligk H, Zechmann CM, Unterhinninghofen R, Kauczor HU, et al. 3D printing based on imaging data: review of medical applications. Int J Comput Assisted Radiol. Surg 2010;5(4):335—41.
[19] Horváth L, Umehara Y, Jud C, Blank F, Petri-Fink A, Rothen-Rutishauser B. Engineering an in vitro air-blood barrier by 3D bioprinting. Sci Rep 2015;5:7974.
[20] Wang X, Yan Y, Zhang R. Recent trends and challenges in complex organ manufacturing. Tissue Eng Part B Rev 2009;16(2):189—97.
[21] Bose S, Vahabzadeh S, Bandyopadhyay A. Bone tissue engineering using 3D printing. Mater Today 2013;16(12):496—504.
[22] Gross BC, Erkal JL, Lockwood SY, Chen C, Spence DM. Evaluation of 3D printing and its potential impact on biotechnology and the chemical sciences. ACS Publications; 2014.
[23] Karoly J, Cyrille N, Francoise M, Keith M, Gordana V-N, Gabor F. Tissue engineering by self-assembly and bio-printing of living cells. Biofabrication 2010;2(2):022001.
[24] Kirchmajer DM, Gorkin Iii R. An overview of the suitability of hydrogel-forming polymers for extrusion-based 3D-printing. J Mater Chem B 2015;3(20):4105—17.
[25] Liu W, Wang D, Huang J, Wei Y, Xiong J, Zhu W, et al. Low-temperature deposition manufacturing: a novel and promising rapid prototyping technology for the fabrication of tissue-engineered scaffold. Mater Sci Eng C 2017;70:976—82.
[26] Marga F, Jakab K, Khatiwala C, Shepherd B, Dorfman S, Hubbard B, et al. Toward engineering functional organ modules by additive manufacturing. Biofabrication 2012;4(2):022001.
[27] Murphy SV, Skardal A, Atala A. Evaluation of hydrogels for bio-printing applications. J Biomed Mater Res Part A 2013;101(1):272—84.
[28] Richards D, Jia J, Yost M, Markwald R, Mei Y. 3D bioprinting for vascularized tissue fabrication. Ann Biomed Eng 2017;45(1):132—47.
[29] Wang S, Lee JM, Yeong WY. Smart hydrogels for 3D bioprinting. Int J Bioprinting 2015;1(1).
[30] Hull CW. Apparatus for production of three-dimensional objects by stereolithography. 1986. Google Patents.
[31] Mandrycky C, Wang Z, Kim K, Kim D-H. 3D bioprinting for engineering complex tissues. Biotechnol Adv 2016;34(4):422—34.
[32] Hospodiuk M, Dey M, Sosnoski D, Ozbolat IT. The bioink: a comprehensive review on bioprintable materials. Biotechnol Adv 2017;35(2):217—39.
[33] Ozbolat IT, Hospodiuk M. Current advances and future perspectives in extrusion-based bioprinting. Biomaterials 2016;76:321—43.
[34] Cengiz IF, Pitikakis M, Cesario L, Parascandolo P, Vosilla L, Viano G, et al. Building the basis for patient-specific meniscal scaffolds: from human knee MRI to fabrication of 3D printed scaffolds. Bioprinting 2016;1:1—10.
[35] Blaeser A, Duarte Campos DF, Weber M, Neuss S, Theek B, Fischer H, et al. Biofabrication under fluorocarbon: a novel freeform fabrication technique to generate high aspect ratio tissue-engineered constructs. BioResearch Open Access 2013;2(5):374—84.
[36] Khalil S, Sun W. Biopolymer deposition for freeform fabrication of hydrogel tissue constructs. Mater Sci Eng C 2007;27(3):469—78.
[37] Cohen DL, Malone E, Lipson HOD, Bonassar LJ. Direct freeform fabrication of seeded hydrogels in arbitrary geometries. Tissue Eng 2006;12(5):1325—35.
[38] Annabi N, Tamayol A, Uquillas JA, Akbari M, Bertassoni LE, Cha C, et al. 25th anniversary article: rational design and applications of hydrogels in regenerative medicine. Adv Mater 2014;26(1):85—124.
[39] Bae S, Kim H, Lee Y, Xu X, Park J-S, Zheng Y, et al. Roll-to-roll production of 30-inch graphene films for transparent electrodes. Nat Nano 2010;5(8):574—8.
[40] Best C, Onwuka E, Pepper V, Sams M, Breuer J, Breuer C. Cardiovascular tissue engineering: preclinical validation to bedside application. Physiology 2016;31(1):7—15.
[41] Arai K, Iwanaga S, Toda H, Genci C, Nishiyama Y, Nakamura M. Three-dimensional inkjet biofabrication based on designed images. Biofabrication 2011;3(3):034113.
[42] Beyer ST, Bsoul A, Ahmadi A, Walus K. 3D alginate constructs for tissue engineering printed using a coaxial flow focusing microfluidic device. IEEE. p. 1206—1209.
[43] Bahk J-H, Fang H, Yazawa K, Shakouri A. Flexible thermoelectric materials and device optimization for wearable energy harvesting. J Mater Chem C 2015;3(40):10362—74.
[44] Yu Y, Zhang Y, Martin JA, Ozbolat IT. Evaluation of cell viability and functionality in vessel-like bioprintable cell-laden tubular channels. J Biomech Eng 2013;135(9):091011.
[45] Zhang Y, Yu Y, Ozbolat IT. Direct bioprinting of vessel-like tubular microfluidic channels. J Nanotechnol Eng Med 2013;4(2):020902.
[46] Ozbolat IT, Chen H, Yu Y. Development of 'Multi-arm Bioprinter'for hybrid biofabrication of tissue engineering constructs. Robotics Computer-Integrated Manuf 2014;30(3):295—304.
[47] Gao Q, He Y, Fu J-z, Liu A, Ma L. Coaxial nozzle-assisted 3D bioprinting with built-in microchannels for nutrients delivery. Biomaterials 2015;61:203—15.
[48] Gao Q, Liu Z, Lin Z, Qiu J, Liu Y, Liu A, et al. 3D bioprinting of vessel-like structures with multilevel fluidic channels. ACS Biomaterials Science & Engineering; 2017.
[49] Jia W, Gungor-Ozkerim PS, Zhang YS, Yue K, Zhu K, Liu W, et al. Direct 3D bioprinting of perfusable vascular constructs using a blend bioink. Biomaterials 2016;106:58—68.
[50] Boland T, Tao X, Damon BJ, Manley B, Kesari P, Jalota S, et al. Drop-on-demand printing of cells and materials for designer tissue constructs. Mater Sci Eng C 2007;27(3):372—6.
[51] Gudapati H, Dey M, Ozbolat I. A comprehensive review on droplet-based bioprinting: past, present and future. Biomaterials 2016;102:20—42.
[52] Xu T, Jin J, Gregory C, Hickman JJ, Boland T. Inkjet printing of viable mammalian cells. Biomaterials 2005;26(1):93—9.
[53] Christensen K, Xu C, Chai W, Zhang Z, Fu J, Huang Y. Freeform inkjet printing

of cellular structures with bifurcations. Biotechnol Bioeng 2015;112(5): 1047–55.

[54] Ozbolat I, Gudapati H. A review on design for bioprinting. Bioprinting 2016;3:1–14.

[55] Saunders R, Bosworth L, Gough J, Derby B, Reis N. Selective cell delivery for 3D tissue culture and engineering. Eur Cells Mater 2004;7(1):84.

[56] Attalla R, Ling C, Selvaganapathy P. Fabrication and characterization of gels with integrated channels using 3D printing with microfluidic nozzle for tissue engineering applications. Biomed Microdevices 2016;18(1):1–12.

[57] Skardal A, Mack D, Kapetanovic E, Atala A, Jackson JD, Yoo J, et al. Bioprinted amniotic fluid-derived stem cells accelerate healing of large skin wounds. Stem cells Transl Med 2012;1(11):792–802.

[58] Cui X, Breitenkamp K, Finn MG, Lotz M, D'Lima DD. Direct human cartilage repair using three-dimensional bioprinting technology. Tissue Eng Part A 2012;18(11–12):1304–12.

[59] Xu T, Binder KW, Albanna MZ, Dice D, Zhao W, Yoo JJ, et al. Hybrid printing of mechanically and biologically improved constructs for cartilage tissue engineering applications. Biofabrication 2012;5(1):015001.

[60] Barron JA, Wu P, Ladouceur HD, Ringeisen BR. Biological laser printing: a novel technique for creating heterogeneous 3-dimensional cell patterns. Biomed Microdevices 2004;6(2):139–47.

[61] Tasoglu S, Demirci U. Bioprinting for stem cell research. Trends Biotechnol 2013;31(1):10–9.

[62] Chang CC, Krishnan L, Nunes SS, Church KH, Edgar LT, Boland ED, et al. Determinants of microvascular network topologies in implanted neovasculatures. Arteriosclerosis, Thrombosis, Vasc Biol 2012;32(1):5–14.

[63] Guillotin B, Souquet A, Catros S, Duocastella M, Pippenger B, Bellance S, et al. Laser assisted bioprinting of engineered tissue with high cell density and microscale organization. Biomaterials 2010;31(28):7250–6.

[64] Guillotin B, Guillemot F. Cell patterning technologies for organotypic tissue fabrication. Trends Biotechnol 2011;29(4):183–90.

[65] Chia HN, Wu BM. Recent advances in 3D printing of biomaterials. J Biol Eng 2015;9(1):4.

[66] Guvendiren M, Lu HD, Burdick JA. Shear-thinning hydrogels for biomedical applications. Soft Matter 2012;8(2):260–72.

[67] Knowlton S, Joshi A, Yenilmez B, Ozbolat IT, Chua CK, Khademhosseini A, et al. Advancing cancer research using bioprinting for tumor-on-a-chip platforms. Int J Bioprinting 2016;2(2).

[68] Ozbolat IT. Scaffold-based or scaffold-free bioprinting: competing or complementing approaches? J Nanotechnol Eng Med 2015;6(2):024701.

[69] Stanton MM, Samitier J, Sánchez S. Bioprinting of 3D hydrogels. Lab a Chip 2015;15(15):3111–5.

[70] Yu Y, Ozbolat IT. Tissue strands as "bioink" for scale-up organ printing. IEEE. p. 1428–1431.

[71] Visscher DO, Farré-Guasch E, Helder MN, Gibbs S, Forouzanfar T, van Zuijlen PP, et al. Advances in bioprinting technologies for craniofacial reconstruction. Trends Biotechnol 2016;34(9):700–10.

[72] Kolesky DB, Homan KA, Skylar-Scott MA, Lewis JA. Three-dimensional bioprinting of thick vascularized tissues. Proc Natl Acad Sci 2016;113(12): 3179–84.

[73] Pati F, Jang J, Ha D-H, Kim SW, Rhie J-W, Shim J-H, et al. Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. Nat Commun 2014:5.

[74] Tan YJ, Tan X, Yeong WY, Tor SB. Hybrid microscaffold-based 3D bioprinting of multi-cellular constructs with high compressive strength: a new biofabrication strategy. Sci Rep 2016:6.

[75] Norotte C, Marga FS, Niklason LE, Forgacs G. Scaffold-free vascular tissue engineering using bioprinting. Biomaterials 2009;30(30):5910–7.

[76] Kucukgul C, Ozler SB, Inci I, Karakas E, Irmak S, Gozuacik D, et al. 3D bioprinting of biomimetic aortic vascular constructs with self-supporting cells. Biotechnol Bioeng 2015;112(4):811–21.

[77] Yu Y, Moncal KK, Li J, Peng W, Rivero I, Martin JA, et al. Three-dimensional bioprinting using self-assembling scalable scaffold-free "tissue strands" as a new bioink. Sci Rep 2016;6:28714.

[78] Kolesky DB, Truby RL, Gladman A, Busbee TA, Homan KA, Lewis JA. 3D bioprinting of vascularized, heterogeneous cell-laden tissue constructs. Adv Mater 2014;26(19):3124–30.

[79] Badhe RV, Bijukumar D, Chejara DR, Mabrouk M, Choonara YE, Kumar P, et al. A composite chitosan-gelatin bi-layered, biomimetic macroporous scaffold for blood vessel tissue engineering. Carbohydr Polym 2017;157:1215–25.

[80] McBeth C, Lauer J, Ottersbach M, Campbell J, Sharon A, Sauer-Budge AF. 3D bioprinting of GelMA scaffolds triggers mineral deposition by primary human osteoblasts. Biofabrication 2017;9(1):015009.

[81] Nishiyama Y, Nakamura M, Henmi C, Yamaguchi K, Mochizuki S, Nakagawa H, et al. Development of a three-dimensional bioprinter: construction of cell supporting structures using hydrogel and state-of-the-art inkjet technology. J Biomechanical Eng 2009;131(3):035001.

[82] Malda J, Visser J, Melchels FP, Jüngst T, Hennink WE, Dhert WJA, et al. 25th anniversary article: engineering hydrogels for biofabrication. Adv Mater 2013;25(36):5011–28.

[83] Hölzl K, Lin S, Tytgat L, Van Vlierberghe S, Gu L, Ovsianikov A. Bioink properties before, during and after 3D bioprinting. Biofabrication 2016;8(3): 032002.

[84] Cubo N, Garcia M, del Cañizo JF, Velasco D, Jorcano JL. 3D bioprinting of functional human skin: production and in vivo analysis. Biofabrication 2016;9(1):015006.

[85] Levato R, Visser J, Planell JA, Engel E, Malda J, Mateos-Timoneda MA. Biofabrication of tissue constructs by 3D bioprinting of cell-laden microcarriers. Biofabrication 2014;6(3):035020.

[86] Chua CK, Yeong WY. Bioprinting: principles and applications. World Scientific Publishing Co Inc; 2014.

[87] Mironov V, Visconti RP, Kasyanov V, Forgacs G, Drake CJ, Markwald RR. Organ printing: tissue spheroids as building blocks. Biomaterials 2009;30(12):2164–74.

[88] Wei J, Wang J, Su S, Wang S, Qiu J, Zhang Z, et al. 3D printing of an extremely tough hydrogel. Rsc Adv 2015;5(99):81324–9.

[89] DeForest CA, Anseth KS. Advances in bioactive hydrogels to probe and direct cell fate. Annu Rev Chem Biomol Eng 2012;3:421–44.

[90] Dolati F, Yu Y, Zhang Y, De Jesus AM, Sander EA, Ozbolat IT. In vitro evaluation of carbon-nanotube-reinforced bioprintable vascular conduits. Nanotechnology 2014;25(14):145101.

[91] Hopley EL, Salmasi S, Kalaskar DM, Seifalian AM. Carbon nanotubes leading the way forward in new generation 3D tissue engineering. Biotechnol Adv 2014;32(5):1000–14.

[92] O'Brien CM, Holmes B, Faucett S, Zhang LG. Three-dimensional printing of nanomaterial scaffolds for complex tissue regeneration. Tissue Eng Part B Rev 2014;21(1):103–14.

[93] Seliktar D. Designing cell-compatible hydrogels for biomedical applications. Science 2012;336(6085):1124–8.

[94] Tibbitt MW, Anseth KS. Hydrogels as extracellular matrix mimics for 3D cell culture. Biotechnol Bioeng 2009;103(4):655–63.

[95] Jia J, Richards DJ, Rodriguez S, Tan Y, Rodriguez J, Visconti RP, et al. Engineering alginate as bioink for bioprinting. Acta Biomater 2014;10(10):4323–31.

[96] Bertassoni LE, Cardoso JC, Manoharan V, Cristino AL, Bhise NS, Araujo WA, et al. Direct-write bioprinting of cell-laden methacrylated gelatin hydrogels. Biofabrication 2014;6(2):024105.

[97] Wang Z, Abdulla R, Parker B, Samanipour R, Ghosh S, Kim K. A simple and high-resolution stereolithography-based 3D bioprinting system using visible light crosslinkable bioinks. Biofabrication 2015;7(4):045009.

[98] Rutz AL, Hyland KE, Jakus AE, Burghardt WR, Shah RN. A multimaterial bioink method for 3D printing tunable, cell-compatible hydrogels. Adv Mater 2015;27(9):1607–14.

[99] Ouyang L, Highley CB, Sun W, Burdick JA. A generalizable strategy for the 3D bioprinting of hydrogels from nonviscous photo-crosslinkable inks. Adv Mater 2017;29(8).

[100] Itoh M, Nakayama K, Noguchi R, Kamohara K, Furukawa K, Uchihashi K, et al. Scaffold-free tubular tissues created by a bio-3D printer undergo remodeling and endothelialization when implanted in rat aortae. PLoS One 2015;10(9): e0136681.

[101] Owens CM, Marga F, Forgacs G, Heesch CM. Biofabrication and testing of a fully cellular nerve graft. Biofabrication 2013;5(4):045007.

[102] Nemeno-Guanzon JG, Lee S, Berg JR, Jo YH, Yeo JE, Nam BM, et al. Trends in tissue engineering for blood vessels. BioMed Res Int 2012;2012.

[103] Skardal A, Zhang J, Prestwich GD. Bioprinting vessel-like constructs using hyaluronan hydrogels crosslinked with tetrahedral polyethylene glycol tetracrylates. Biomaterials 2010;31(24):6173–81.

[104] Wang C, Cen L, Yin S, Liu Q, Liu W, Cao Y, et al. A small diameter elastic blood vessel wall prepared under pulsatile conditions from polyglycolic acid mesh and smooth muscle cells differentiated from adipose-derived stem cells. Biomaterials 2010;31(4):621–30.

[105] Zhao X, Liu L, Wang J, Xu Y, Zhang W, Khang G, et al. In vitro vascularization of a combined system based on a 3D printing technique. J Tissue Eng Regen Med 2016;10(10):833–42.

[106] Skardal A, Zhang J, McCoard L, Oottamasathien S, Prestwich GD. Dynamically crosslinked gold nanoparticle–hyaluronan hydrogels. Adv Mater 2010;22(42):4736–40.

[107] Gao Q, Liu Z, Lin Z, Qiu J, Liu Y, Liu A, et al. 3D Bioprinting of Vessel-like Structures with Multi-level Fluidic Channels. ACS biomaterials science & engineering; 2017.

[108] Tan Y, Richards DJ, Trusk TC, Visconti RP, Yost MJ, Kindy MS, et al. 3D printing facilitated scaffold-free tissue unit fabrication. Biofabrication 2014;6(2):024111.

[109] Bertassoni LE, Cecconi M, Manoharan V, Nikkhah M, Hjortnaes J, Cristino AL, et al. Hydrogel bioprinted microchannel networks for vascularization of tissue engineering constructs. Lab a Chip 2014;14(13):2202–11.

[110] Miller JS, Stevens KR, Yang MT, Baker BM, Nguyen D-HT, Cohen DM, et al. Rapid casting of patterned vascular networks for perfusable engineered three-dimensional tissues. Nat Mater 2012;11(9):768–74.

[111] Ovsianikov A, Gruene M, Pflaum M, Koch L, Maiorana F, Wilhelmi M, et al. Laser printing of cells into 3D scaffolds. Biofabrication 2010;2(1):014104.

[112] Wu W, DeConinck A, Lewis JA. Omnidirectional printing of 3D microvascular networks. Adv Mater 2011;23(24).

[113] Lee VK, Kim DY, Ngo H, Lee Y, Seo L, Yoo S-S, et al. Creating perfused functional vascular channels using 3D bio-printing technology. Biomaterials 2014;35(28):8092–102.

[114] Kang H-W, Lee SJ, Ko IK, Kengla C, Yoo JJ, Atala AA. 3D bioprinting system to produce human-scale tissue constructs with structural integrity. Nat Biotech 2016;34(3):312–9.

[115] Kinstlinger IS, Miller JS. 3D-printed fluidic networks as vasculature for engineered tissue. Lab a Chip 2016;16(11):2025–43.

[116] Lee VK, Lanzi AM, Ngo H, Yoo S-S, Vincent PA, Dai G. Generation of multi-scale vascular network system within 3D hydrogel using 3D bio-printing technology. Cell Mol Bioeng 2014;7(3):460–72.

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

[118] Ozbolat IT. Bioprinting scale-up tissue and organ constructs for transplantation. Trends Biotechnol 2015;33(7):395–400.