REVIEW ARTICLE

Progress in organ 3D bioprinting

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Abstract: Three dimensional (3D) printing is a hot topic in today's scientific, technological and commercial areas. It is recognized as the main field which promotes "the Third Industrial Revolution". Recently, human organ 3D bioprinting has been put forward into equity market as a concept stock and attracted a lot of attention. A large number of outstanding scientists have flung themselves into this field and made some remarkable headways. Nevertheless, organ 3D bioprinting is a sophisticated manufacture procedure which needs profound scientific/technological backgrounds/knowledges to accomplish. Especially, large organ 3D bioprinting encounters enormous difficulties and challenges. One of them is to build implantable branched vascular networks in a predefined 3D construct. At present, organ 3D bioprinting still in its infancy and a great deal of work needs to be done. Here we briefly overview some of the achievements of 3D bioprinting technologies in large organ, such as the bone, liver, heart, cartilage and skin, manufacturing.

Keywords: organ; 3D bioprinting; bone; heart; liver; cartilage; skin

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1. Introduction

It is widely believed that there are 79 organs in the human body[1]. Each of the organs consists of different tissues. Most of the tissues in the organs have heterogeneous structures that confers one or more highly-specific functions. Organs can be divided into several groups, such as sensory, internal and structural, according to their main functions[2]. The sensory organs include the eyes, nose, ears and tongue. The internal organs (also known as viscera) include the liver, lung, kidney, heart, esophagus, stomach and bowel, while the structural organs include the bones, cartilages and muscles.

With the advancement in modern science and technology, organ failure or deterioration caused by acute/chronic diseases, congenital malformations and traffic accidents have become one of the huge social problems[3]. According to the statistics, there are about 1.5 millions of patients who require organ transplantations in China every year, but only less than 1% of patients can obtain suitable organs[4]. Compared to the traditional artificial organs made from polymers or metals, bioartificial organs made from living cells and biomaterials have become more and more prevalent.

Currently, a variety of bioprinting strategies have been developed to tackle the challenges for manufacturing bioartificial organs with physiological functions[5–8]. A main character of these strategies is to build complex organ geometries *via* spatiotemporal pattern of heterogenoustypes of "bioinks", especially cells. These strategies can be classified into three main groups: multi-nozzle rapid prototyping (MNRP), decellularization organ regeneration and combined mold system. Each of them has its own

advantages and disadvantages in bioartificial organ manufacturing areas[9–11]. An obvious advantage of the MNRP technology is that it can produce bioartificial organs automatically mimicking their natural counterparts using heterogeneous cell types and other biomaterials. In this article, we highlight some of the three-dimensional (3D) achievements of various bioprinting technologies in five large organs, including the bone, liver, heart, cartilage and skin, manufacturing.

2. Different Types of Bioprinting

Given that working principles, five major types of 3D bioprinting technologies include inkjet-based bioprinting, extrusion-based bioprinting, laser-assisted bioprinting, stereolithography-based bioprinting and microvalve-based bioprinting[12–14]. Among these technologies extrusion-based bioprinting technologies have been widely used to build cell-laden 3D tissues and organs.

2.1 Inkjet-based Bioprinting

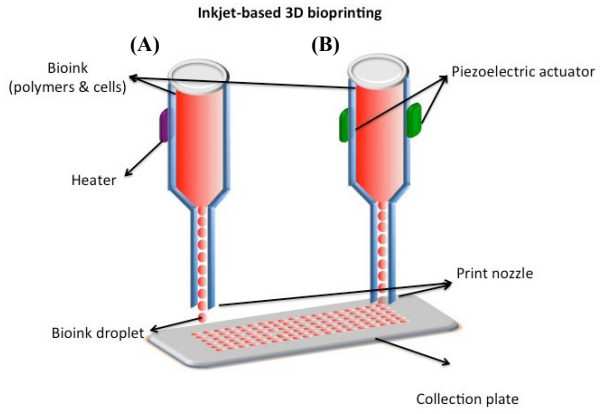

Figure 1. Schematic diagram of inkjet-based bioprinting (**A**: Heater; **B**: Piezoelectric actuator)

Inkjet-based bioprinting initially employed a commercial printer to spray cells (Figure 1)[15]. Inkjet bioprinters, known as droplet-based bioprinters, use thermal or acoustic force to eject liquid drops onto a substrate and build constructs layer-by-layer. In thermal inkjet bioprinting, "bioink" droplets are generated by electrically heating the print head to force cells in the liquid drops out of nozzle by increasing pressure[16]. Bioinks made of cells, scaffold materials and growth factors can be deposited accurately through controlling the droplet size and deposition rate[17]. During the inkjet bioprinting process, the heating temperature can reach approximate 300 °C. However, it lasts for very short of duration, resulting in the system temperature raising 4–10 °C with no obvious detrimental effect on cells. In piezoelectric inkjet bioprinting, bioink droplets are generated by acoustic wave induced by piezoelectric crystal inside the print head.

The advantages of inkjet-based bioprinting in organ 3D bioprinting contain the fast response speed, the high formation precision, and the high efficiency. These can be analysed through two aspects. On the one hand, the acoustic 3D bioprinters can be well-controlled through adjusting the jetting direction, droplet size, and cell viability. On the other hand, the thermal bioprinters can be well-controlled through adjusting the printing speed and cost. One obvious drawback of inkjet bioprinting in organ 3D bioprinting is that the "bioinks" should be in liquid forms with low viscosities[13]. This has greatly limited the height of the constructs. Only low concentration of polymeric bioinks with a low cell density (fewer than 10^6 cells/mL) can avoid nozzle clogging and reduce shear stress on cells[15–17]. Another obvious drawback of inkjet bioprinting in organ 3D bioprinting is the poor mechanical properties of the 3D constructs. Till now, most of the researchers in this field do their studies by modifying commercial inkjet printing systems to print living cells. This has greatly limited their development in soft and hardware as well as the complexity of printed constructs. Due to these drawbacks, inkjet-based bioprinting is still in its infancy stage for large organ 3D bioprinting whereas extrusion-based bioprinting has been prevalently used for numerous studies.

2.2 Extrusion-based Bioprinting

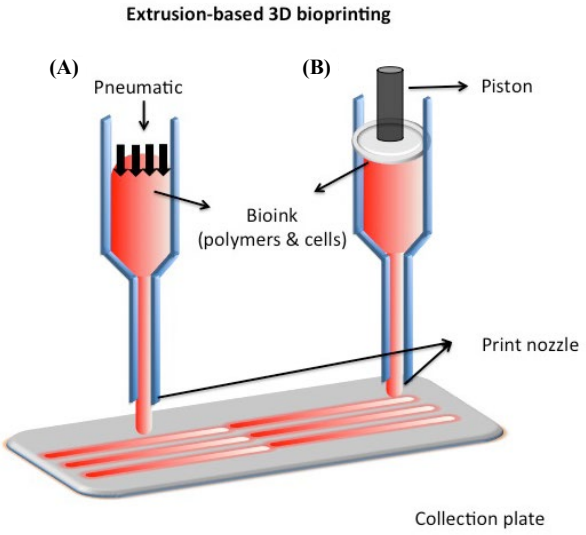

Figure 2. Schematic diagram of extrusion-based bioprinting (**A**: Pneumatic; **B**: Piston)

Extrusion-based bioprinting is a particular deposition process using fluidic polymeric solutions or hydrogels as bioinks (Figure 2)[18–24]. The extrusion-based bioprinters are normally consisted of a three-axis automatic extrusion system equipped with a fluid-dispensing nozzle (or head)[25–28]. During the extrusion processes, cell-laden bioinks are deposited in cylindrical filaments under the control of a computer-aided designing (CAD) model. At present, it is the only technology that can produce large scale-

Table 1. Comparison of different bioprinting techniques for organ manufacturing

Technique	Pros	Cons	References
Inkjet-based	High printing resolution (~20 μm); Several thermosensitive hydrogels can be printed; Simple sample-loading requirements; Low viscosity of cell suspensions (up to 10^6 cells/mL) or cell-laden hydrogels (3–30 mPs); Middle cell viability (> 70%).	Limited materials can be used; Complex 3D constructs are difficult to achieve; Limited height (< 10 μm); Potential cell desiccation; High shear stress endured by cells; Droplet instability at high printing speed; Poor cell sedimentation effects; Poor mechanical properties.	[13–17]
Extrusion-based	Easy updated soft and hardware; Flexible geometric shapes; Multiple biomaterials including cell types can be incorporated; Homogeneous and heterogeneous structures can be created; Good cell sedimentation effect; High cell viability (> 98%).	Material viscosity and temperature dependent; High viscosity hydrogels may affect cell activities.	[18–24]
Laser-assisted	Relatively high printing resolution (~40 μm); Wide range of printable viscosity; High cell viability (>90%).	High cost; Low efficiency; Difficult to incorporate multiple bioactive agents; Poor cell sedimentation effects; Poor cell homogeneity.	[39–42]
Stereolithography-based	Several photopolymerized materials can be used; High building velocity and accuracy; Multiple hydrogels can be printed simultaneously.	Cytotoxic of the laser beam and photo-initiators; Additional post-curing process may be necessary to remove the unpolymerized liquid resin; Poor cell sedimentation effects.	[43–46]
Microvalve-based	Relatively high printing resolution (~150 μm); Low viscosity of hydrogels (1–70 mPs); middle cell viability (> 80%); Middle cell sedimentation effect.	High shear stress suffered by cells; weak mechanical properties.	[50–53]

up cell-laden constructs containing both micro-/macro physiological environments in a controllable manner. Heterogeneous tissues and organs can be manufactured (*i.e.* produced) using either a single-nozzle 3D bioprinter with stem cells/heterogenous growth factors or a multi-nozzle 3D bioprinter with multiple cell lineages.

For extrusion-based bioprinting, the enabling 3D printers and biocompatible polymers are two major factors (*i.e.* elements) affecting the final 3D constructs. The resolution, shape and quality of the 3D constructs are mainly determined by the printability of the polymeric solutions or hydrogels, which has non-consistency with the cell viability. The viscosity of some of the polymeric "bioinks" may decrease when the shear stress of the printing system is increased. This may help to protect the cells and improve the resolution of the 3D constructs[29–34].

The advantages of extrusion-based bioprinting in organ 3D bioprinting include high cell densities, large 3D constructs and fast printing speeds. Beside polymeric solutions or hydrogels, extracellular matrices (ECMs) and cell aggregates can also be used as bioinks. The disadvantage of extrusion-based bioprinting in organ 3D bioprinting is that there are limited polymeric solutions or hydrogels that have good biocompatibilities and can be printed into large constructs in layers[35–38].

2.3 Laser-assisted Bioprinting

Laser-assisted bioprinting is based on the laser pulse to generate a high-pressure bubble between a solution and a piece of glass containing cells towards the collective substrate (Figure 3)[39,40]. It can produce micro cell-laden 3D constructs with a range of viscosities (1–300 mPa/s) of polymers in a high resolution[41,42].

The advantage of laser-assisted bioprinting in organ 3D bioprinting includes avoiding the problems of nozzle clogging with cells and/or polymeric biomaterials. The disadvantage of laser-assisted bioprinting in organ 3D bioprinting is the high cost of the laser-assisted 3D bioprinters.

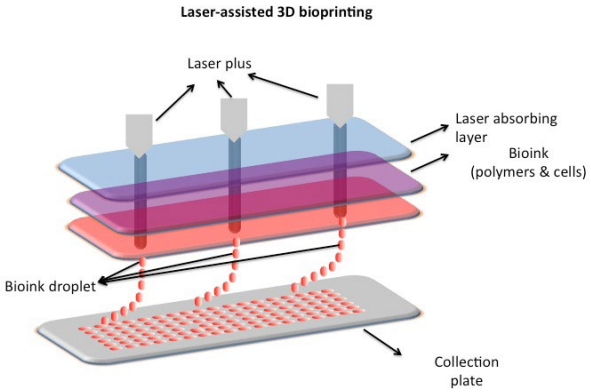

Figure 3. Schematic diagram of laser-assisted bioprinting

2.4 Stereolithography-based Bioprinting

Stereolithography (STL) technology is a solid free-form, nozzle-free technology based on photo-sensitive

macromolecule (or polymer) formulation[43]. It is a multi-layer procedure through the selective photo-initiated curing reaction of a low-molecular weight pre-polymer, additives and photo-initiators. Either a focused ultraviolet beam light or a mask-based irradiation can be used to selectively solidify the liquid photopolymer. Both single-photon polymerization and two-photon polymerization (2PP) can be induced at the printing stage[44]. A number of biomaterials can be added in the STL printing process. Optimal digital micromirror devices can work with wavelengths between 385–405 nm with expected lifetime of 2,000 h when exposed to a radiation with light intensities of 10 w/cm^2. Light-sensitive polymer hydrogels, such as hyaluronic acid, collagen, chitosan, diacrylate (PEGDA), containing cells can also be printed using these devices in a layer-by-layer manner[45,46]. The advantage of stereolithography-based bioprinting in organ 3D bioprinting is the high building velocity and accuracy. The disadvantages of stereolithography-based 3D bioprinting in organ 3D bioprinting is the high cost of the devices, and the cytotoxicity of the lights and photo-initiators[47–49].

2.5 Microvalve-based Bioprinting

Similar to inkjet-based bioprinting, microvalve-based bioprinting is a drop-on-demand technology. It comprises a three-axis movable robotic platform and an array of electromethanical microvalve heads[50]. Each of the microvalve head is connected to an individual gas regulator with pneumatic pressure. Liquid "bioinks" can be deposited when the pneumatic pressure overcomes the fluid viscosity and surface tension at the open orifice[51,52]. Cell viability and sedimentation effect during the printing process are the major issues in most of these bioprinting systems.

The main advantages of microvalve-based bioprinting in organ 3D bioprinting are the synchronized ejection of biomaterials including cells from different microvalve heads, the thin deposition layers (1–2 μm thickness), and the high throughput printing velocity (≈ 1000 droplets per second). The disadvantage of microvalve-based bioprinting in organ 3D bioprinting is that it can only print hydrogels within a limited range of viscosities (*e.g.* 1–200 mPa) and cell concentrations (up to 10^6 cells/mL)[53]. Cell viability and sedimentation effect depend largely on the employed liquid polymeric "bioinks".

No matter which bioprinting technology is applied in organ 3D bioprinting, good biocompatibility (or cytocompatiblity) of the polymeric solutions or hydrogels is a prior requirement for a successful 3D printable bioink, not only for the printing process, but also for the post-printing procedures, such as solvent exchanging, chemical crosslinking and polymeric degradation. The balance between a high cell viability and the physiological functionality realization of a supportive polymeric solution or hydrogel often need to be addressed before the 3D bioprinting process.

3. Large Organ 3D Bioprinting

A bone is a distinct rigid organ that constitutes part of the vertebrate skeleton (Figure 4)[54,55]. It is mainly composed of osteoblasts, osteoclasts and hard extracellular matrices (ECMs), such as collagen and hydroxyapatite. The bone has multiple functions, such as to support and protect various organs, produce red and white blood cells, store minerals, and enable mobility[56]. In the human body, different bones appear in a variety of shapes and sizes and have an intricate internal and external structure. These bones can be classified into five types: long, short, flat, sesamoid and irregular. There are blood vessels and marrow channels in the long bones which are difficult for the ordinary processing technologies to construct. Some large bones, such as the skull, radius and tibia, have complex shapes and contours. The contours may be strong angles, slightly concave or slightly convex, which need specific processing technologies to complete[57–60].

As early as in 1989, Madison first used rapid prototyping (RP) technology to diagnose bone diseases[61]. In 1998, Iseri *et al.* obtained a skull model of a 12-year-old girl using reverse engineering[62]. At the initial stage when RP technology was employed in 3D printing, researchers focused on matching the mechanical properties of bone *via* printing synthetic polymers to make 3D bone regenerative scaffolds. In 2002, Cheung *et al.* built a patient's maxillofacial region using the RP technique to provide a clear picture to guide the operation[63]. From then, various polymers in different states, such as thread, granular, solution, hydrogel, or slurry, were printed into porous structures under the instruction of CAD models. The porous scaffolds provided a favourable environment for cells to grow in. These works have provided a primary basis for large bone 3D bioprinting using either fused deposition modeling, extrusion-based or stereolithography-based printing technologies.

In recent years, a variety of 3D printing technologies have been further developed to construct bone repair

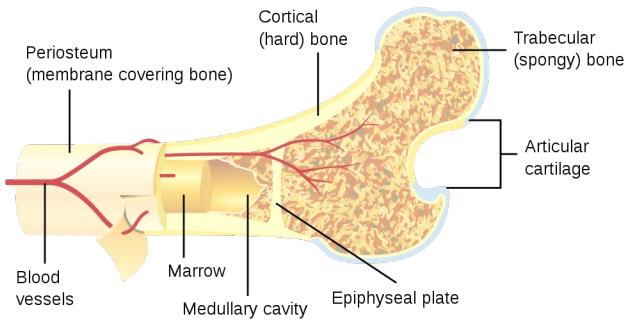

Figure 4. Cross-section of a large bone

materials mimicking the composition of bone tissues and the microenvironment of bony ECMs. For example, in 2002, Ang *et al.* at National University of Singapore printed a mesh hydroxyapatite-chitosan structure as bone repair fillers[64]. In 2005, Seitz *et al.* at Germany cooperated with Generis GmbH (Augsburg, Germany) company developed a 3D printed ceramic bone repair material[65]. In 2008, Kouhi et *al. at* Australia Swinburne University of Technology prepared a P400ABS plastic jawbone by fused deposition manufacturing[66]. In 2010, Smith *et al.* at nScrypt company in Orlando produced a hard tissue repair material using titanium and caprolactone[67]. In the same way, Lee *et al.* printed a porous calcium phosphate cement/alginate scaffold by depositing a solution of α-tricalcium phosphate-based powder and sodium alginate in a calcium chloride bath[68]. Comparing with the traditional metal or polymethyl methacrylate (mechanical and semi-mechanical) bone repair materials, most of the 3D printed bone repair materials have two obvious characteristics: one is made of biodegradable polymers, and the other is having go-through channels or pores. The predefined channels in the 3D printed construct are useful for nutrient supply and metabolite elimination for the in-growth of osteoblasts[69–73]. Some of the bone repair materials have showed good osteogenic effects and bone formation capabilities.

For large bone repair, a great deal pioneering work has been done in Tsinghua University using extrusion-based 3D printing technologies. Some ceramic materials, such as hydroxyapatite (HA) and beta-tricalcium phosphate (β-TCP), were incorporated into synthetic poly (lactic-co-glycolic acid) (PLGA) or poly-lactide (PLA) scaffolds to promote osteogenesis. Other biomaterials, such as collagen and bone growth factors could also be incorporated[76]. For example, In 2000, Yan *et al.* used a single nozzle low-temperature RP technology to prepare large bone repair materials with predefined (go-through) channels 200–500 μm in diameter which were hard to produce using traditional manufacturing technologies (Figure 5)[77]. Large scale-up cylindrical or grid PLA/HA or PLGA/HA scaffolds were produced for defect bone tissue regeneration. Similar research works were performed by other groups in American and Singapore with different biomaterials[78–80]. In 2009, Professor Wang in this group cooperated with Professor Qin in the Chinese University of Hong Kong constructed a large dual-functional bone repair material consisting of P-chitosan and S-chitosan through their home-made double-nozzle low-temperature deposition 3D bioprinter[81]. Multiple biochemical factors were entrapped in the synthetic polymeric scaffolds with precise predesigned (or predefined) patterns (or channels). Later in 2010, six mandible injury patients in Zhongshan People's Hospital were treated with the related 3D printed bone repair materials[82]. Multiple functional bone repair

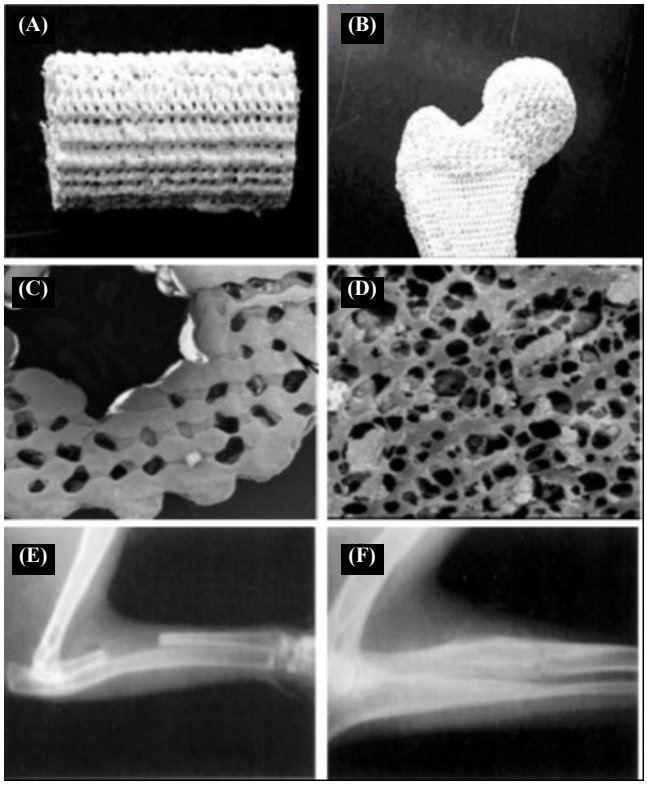

Figure 5. 3D bioprinted large bone repair materials for canine radius repairment, made of PLA (or PLGA)/HA with predefined internal morphology and macroscopic shapes.

materials with gradient structures were produced. The predefined channels could recapitulate the natural bony tissue microenvironment and promote the body fluid to diffuse. Nevertheless, most of the early 3D printed bone repair materials are made of synthetic polymers with no living cells involved in the 3D printing processes. These materials could act as bone tissue regenerative temporaries to promote cells growing in but not the real natural organ mimicking substitutes.

Compared with other organs, the composition of the bone is relatively simple and it is easy to be simulated. Until now, there are many reviews on this subject[83–89]. Numerous studies have focused on producing 3D printed bone regenerative scaffolds (or substitutes) in a custom-designed manner[90,91]. Most of the scaffolds are made of synthetic polymers, such as PLGA, polycaprolactone (PCL), with good mechanical properties, and ceramic materials, such as hydroxyapatite and beta-tricalcium phosphate (β-TCP)[92–95]. For example, in 2016 Jakus *et al.* developed an elastic construct for bone regeneration. They dissolved PCL or PLGA and HA in a trisolvent mixture as the printable "bioink". The printed 3D constructs can be handled versatilely, such as cutting, folding, rolling and suturing. Human mesenchymal stem cells (MSCs) seeded on the 3D constructs showed a significant up-regulation of pro-osteogenic genes,

collagen type I, osteocalcin, and osteopontin at day 28. When the 3D constructs were implanted in a macaque calvarial defect for 4 weeks, excellent new bone formation accompanying with the vascularization and integration of surrounding tissue[96]. At the same time, La *et al.* reported a bone substitute that replicates the micro- and mineralized environment through printing PCL/PLGA/TCP scaffolds, and then coating them with the bone dECM (bdECM) that was extracted from bovine tibiae. The PCL/PLGA/TCP/bdECM scaffolds exhibited significantly enhanced osteogenic gene expression and calcium deposition. These experiments have further certified the bone regenerative effects of the PLGA/HA scaffolds which have been printed more than ten years ago in Professor Wang's groups.

3.2 Liver 3D Bioprinting

The liver is a vital visceral organ in the human body (Figure 6). Unlike the structural organ bone, liver 3D bioprinting has several bottleneck problems to solve: one of them is how to construct the branched vascular and bile duct networks, another of them is how to distribute more than three cell types in a predefined 3D construct with a high cell density and make them develop to functional tissues[7].

There are several CAD models have been used to construct bioartificial livers. Some of the CAD models are made of experience. For example, in 2004 professor Wang and co-workers first assemble cell-laden gelatin-based hydrogels into large scale-up liver tissues with predefined structures (go-through channels) using a extrusion-based 3D printing system under the instruction of an experiential CAD model[16–19]. The predefined structures were printed via a pressure-controlled syringe. This technique allows the deposition of cell-laden hydrogels solutions with high concentration and velocity. Cylindrical channels with diameters ranging from 100 to 300 μm were produced. After 3D printing, the gelatin-based polymers in the cell-laden constructs were submitted to a chemical crosslinking process to stabilize the structures and improve the mechanical properties. Hepatocytes encapsulated in the gelatin-based hydrogels remained viable and produced hepatic ECMs during the 8 weeks' *in vitro* culture. This is a great breakthrough in tissue engineering field which has encountered numerous bottleneck problems in organ manufacturing areas. Thus difficult problems, such as large tissue formation and nutrient supply, have been solved therefore. In 2007, a large scale-up vascularized liver tissue was first produced in the same group using another experiential CAD model[29,30]. From then, actual bioartifical organ manufacturing has been put forward and developed very quickly. In 2009, a 3D printed complicated organ with a whole fluent of endothelial layer covered the inner channels of vascular network was produced[25–28]. It was possible to observe that endothelial cells aligned inside the surface of the predefined channels. More than three cell types formed functional tissues in a complex 3D construct. This technique has advanced other researches at least ten years in organ manufacturing areas[97,98].

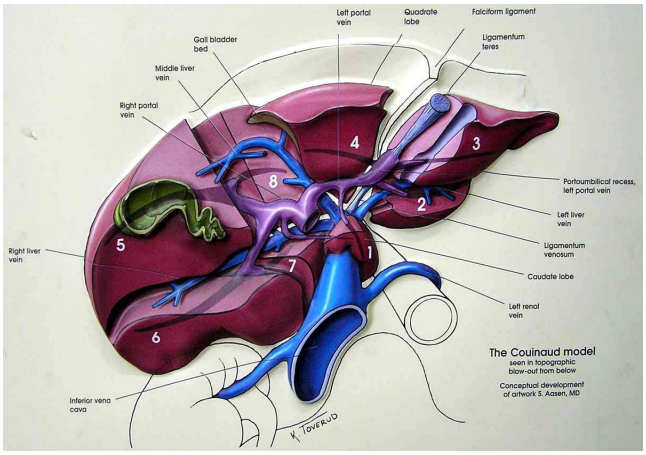

Figure 6. Schematic description the complex structure of the liver

At the same time, other groups throughout the world still devoted themselves in tissue engineered organ dreams with their porous scaffolds. For example, Huang *et al.* seeded hepatoma cells on a 3D printed branched vessel network which consists of avidin and biotin in 2007[99]. This is a typical traditional tissue engineering method to manufacture complex organs with a porous scaffold. Later in 2013, Organovo company in American printed a micro liver-tissue mimicking the techniques developed in Professor Wang' group. According to the British New Scientist magazine website report, the micro-liver tissue, 0.5 mm in thickness, 4 mm in square size, was created. To build the micro liver-tissue, two main cell types of the liver, *i.e.* hepatocytes and hepatic stellate cells, were printed into 20 layers[100]. Cells in the micro liver-tissue can survival for more than five days. Neovascularization played a role in the cell survival capabilities. In 2014, a bioartificial liver containing both vascular and nervous networks has been produced layer-by-layer using a combined MNRP under the instruction of a much more complex experienced CAD model[101]. The potential of this technology will eventually facilitate the manufacture of bioartificial livers, and make the liver 3D bioprinting an impending reality.

Currently, there is a trend that to make the CAD models from clinical patients. For instance, some current clinical diagnostic technologies, such as computer tomography (CT) and magnetic resonance imaging (MRI), have been explored to acquire liver image information of the patients. The CT and MRI image information are subsequently transformed into CAD models (*i.e.* liver manufacturing blueprints) and segregated into 2D horizontal slices to provide instructions to the 3D bioprinters.

For liver 3D bioprinting, two essential elements should be addressed: (1) a extrusion-based multi-nozzle 3D bioprinter with an appropriate soft/hardware. (2) multiple cell lineages from the liver or stem cells with proper growth factors. The extrusion-based multi-nozzle 3D bioprinter can print multiple cell types along with other biomaterials simultaneously in a layer-by-layer manner, which offers a great opportunity in manufacturing the complicated bioartificial livers with more than 6 cell types or tissues[102–106]. These technologies allow to use multiple polymeric hydrogels and growth factors to control the spatial distribution of cells and bioactive agents.

3.3 Heart 3D Bioprinting

The heart is one of the most important internal organ of human beings (Figure 7). It is composed of three different cardiac tissues: myocardium, pericardium and endocardium. The myocardium is the thick muscular layer of the heart wall which consists of cardiomyocytes, aligning themselves in an anisotropic manner and promoting the electrical activation of the cardiac muscles, and taking up to 30%–40% of the entire cell population. The pericardium is a conical, flask-like, double-wall fibroserous sac that encloses the blood vessels from the root of the heart. The endocardium is the endothelial lining of the innermost heart chambers and heart valves. It is primarily made up of endothelial cells that seal the heart and connect the surrounding blood vessels[107,108]. While the rest cell types of the heart are mainly non-myocyte fibroblasts[109]. The elasticity of the cardiomyocytes and their collagen-based ECMs in a normal heart are pliable and tough enough to generate actomyosin forces and pump the heart.

At present, there is limited literature for the whole heart 3D bioprinting. A number of 3D printing techniques have been developed to improve the functionality of the cardiac tissues. For example, in 2007, Marga *et al.* emitted a stream of cell-laden hydrogel microparticles in a well-defined topological pattern to form 3D myocardial patches using a inkjet-based bioprinting technique[110]. This technique is supported by the self-assembly and self-organizing capabilities of cells. In 2011, Gaebel *et al.* patterned human stem cells and endothelial cells with laser printing for cardiac regeneration[111]. In 2012, human cardiomyocyte progenitor cells (HCMPCs) in alginate hydrogel was printed by the same group[112]. HCMPCs in the alginate hydrogel showed an increase of cardiac commitment while at the same time maintaining viability and proliferation. In 2013, Duan *et al.* constructed trileaflet valve like conduits using sinus smooth muscle cells (SMCs) and alginate/gelatin hydrogels[113]. Cell viability in the alginate/gelatin hydrogels attained 81.4%. In 2014, similar study was carried out in the same group using human aortic vascular interstitial cells (HAVICs) in methacrylated hyaluronic acid (MeHA) or gelatin methacrylate (GelMA) hydrogels[114]. High HAVIC viability of the encapsulated cells (>90%) and promising remodeling potentials were obtained using this technology. The main concern of this technology is that polymethacrylate is an unbiodegradable polymer. It may hinder the cells to form functional tissues during the later cultures. In 2015, Hinton *et al.* created a heart CAD model using a reversible freeform embedding hydrogel[115]. A extrusion-based 3D bioprinting technology was used to produce a functional cardiac tissue, and particularly, a semilunar heart valve with three main components: a relatively stiff heart valve root populated by contractile SMCs, three thin flexible leaflets contain fibroblastic interstitial cells and three sinuses[116]. The semilunar heart valve can allows blood to be forced into the arteries and prevent the backflows. Hybrid hydrogel properties were studied by changing concentrations of the two compositions: MeHA and GelMA. The optimized hydrogel formulation was mixed with HAVICs. After 7 days in static culture, the 3D bioprinted valve showed well maintained structure, high viability of the encapsulated cells (> 90%), as well as promising remodeling potentials. In 2006, Chang *et al.* at the Cardiovascular Innovation Institute provided several sets of baseline parameters according to the different humidity of Pluronic F127 hydrogel for direct-write printing of the biomaterial, which was hoping to be used in heart tissue 3D bioprinting[117].

It should be aware of that either the semi-aortic valve or whole heart replacement is a dangerous procedure (a high-risk operation). Until present, the bioprinted aortic valve cannot open and close by itself without the presence of the rest of the heart. One reason is that the cardiac muscle cells are terminally differentiated cells that have no capability to regenerate and form new cardiac tissues. A number of techniques have thus been

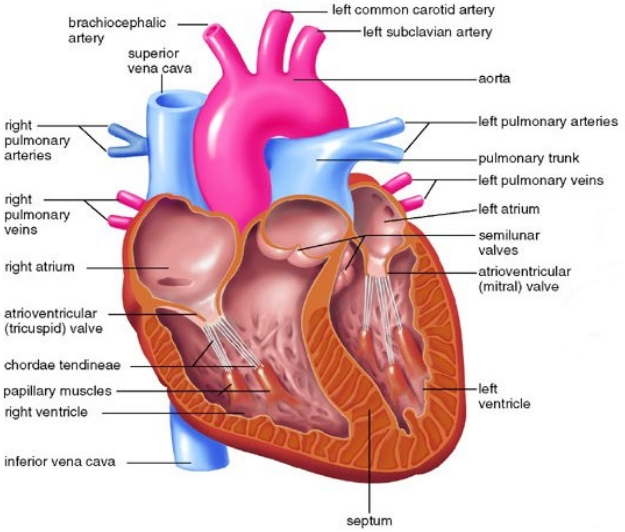

Figure 7. Schematic description of the heart

developed to improve the functionality of engineered cardiac tissues. For example, to increase the mechanical properties, Hasan *et al.* developed an *in vitro* cell culture system to stimulate the physiological pressure and flow of the heart valve[118–120]. This stimulation could improve the strength of the heart valve before a possible implantation. A bioreactor system has been used to train printed heart valves, which could be beneficial for in vitro testing and maturation. Much more work needs to be done before the 3D printed bioartificial hearts to be applied clinically[121].

3.4 Cartilage 3D Bioprinting

Cartilage is a resilient and smooth elastic organ of the body, which protects the ends of long bones at the joints (*e.g.* the elbows, knees and ankles) (Figure 8). It is a structural component, made up of specialized cells called chondrocytes, of the rib cage, the ear, the nose, the bronchial tubes or airways, the intervertebral discs, and many other body components. The chondrocytes produce large amounts of ECM composed of proteoglycan, collagen and elastin fibers. Especially, there are no blood vessels in cartilage to supply the chondrocytes with nutrients. It is not as hard and rigid as bone, but it is much stiffer and much less flexible than muscle[123]. Like many other organs, cartilage exhibits multiple zonal organizations with highly coordinated cell distribution.

Cartilage can be categorized into three types: (1) hyaline cartilage with low-frication and wear-resistant properties; (2) elastic cartilage with flexible property; (3) fibrocartilage with tough and inflexible properties. Due to the lack of blood vessels, cartilage grows and repairs more slowly than other tissues/organs.

Through the research of cartilage regeneration is nearly as early as those in the bone, 3D printing technologies which have been used in cartilage regeneration is relatively late. For example, in 2014,

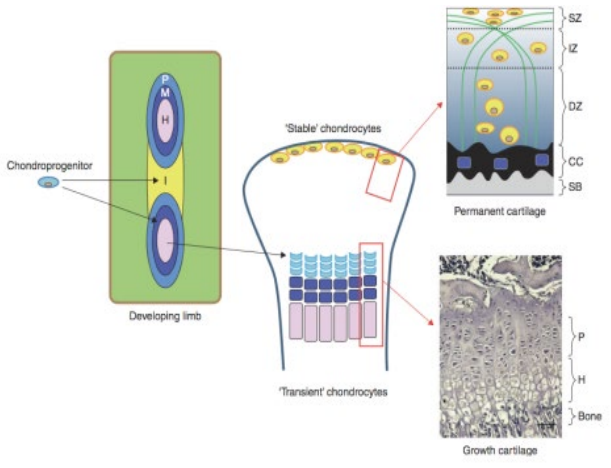

Figure 8. Schematic diagram of the developmental origins of articular and growth plate cartilage[122]

Lee *et al.* printed a meniscus scaffold with two different zones: the white zone, which is located at the inner zone of the meniscus, consists of chondrocyte-like cells with abundant collagen type II and glycosaminoglycans (GAG), whereas the red zone, which is in the other zone of the meniscus, contains fibroblast-like cells with collagen type I[124]. Human connective tissue growth factor and transforming growth factor β3 were then placed in the red and white zones respectively. The two zones spatiotemporally released the growth factors and induced the human synovium smooth muscle cells to form a zone-specific matrix, *i.e.* collagen type II in the white zone and collagen type I in the red zone. The zone-specific phenotypes were further exhibited in a 3-month implantation of a sheep partial meniscectomy model.

In 2015, Kundu *et al.* printed a hybrid cartilage construct containing chondrocyte, alginate, and PCL[125]. In 2016, the same group developed an autologous cartilage construct consisting of autologous chondrocyte, alginate, and PCL for auricular reconstruction[126]. The synthetic PCL was printed with alginate hydrogel and cells, which can provide the construct with long-term stability. The rigid properties of PCL may induce abrasion of the surrounding cartilage tissue. In the same year, Hung *et al.* fabricated a biodegradable polyurethane (PU) involving cartilage construct which exhibited a high strain recovery property[127]. Other bioactive compounds, such as hyaluronic acid and growth factors, can be encapsulated into the PU "bioink" and induce high GAG secretion at 4 weeks after implantation into rabbit osteochondral defects. The formation of cartilage was observed by safranin-O staining.

3.5 Skin 3D Bioprinting

The skin is the largest organ in the human body, which is accounting for about 15% body weight and maintains the body's temperature through sweat or other mechanism (Figure 9)[128]. Along with sweat glands, the skin contains oil glands to keep the skin from drying out and the hair from becoming brittle. The skin consists of three layers namely epidermis, dermis and hypodermis. Epidermis is the outer layer, consisting of keratinocytes (KCs), dermis is the middle layer, consisting of collagen and fibroblasts, hypodermis is the inner layer, consisting of lipocytes and collagen. There are about 19 million skin cells in every square inch of the human body! Although numerous studies have tried to generate full-thickness skin substitutes, most methods are dependent on the technique that seed cells on a porous scaffold, with which it is not easy to recapitulate the heterogeneity of skin comprising multiple types of cells. 3D bioprinting allows similar skin geometry to be built *via* the spatiotemporal pattern of the related cell types of the skin[129].

Traditional skin substitutes either are made of natural

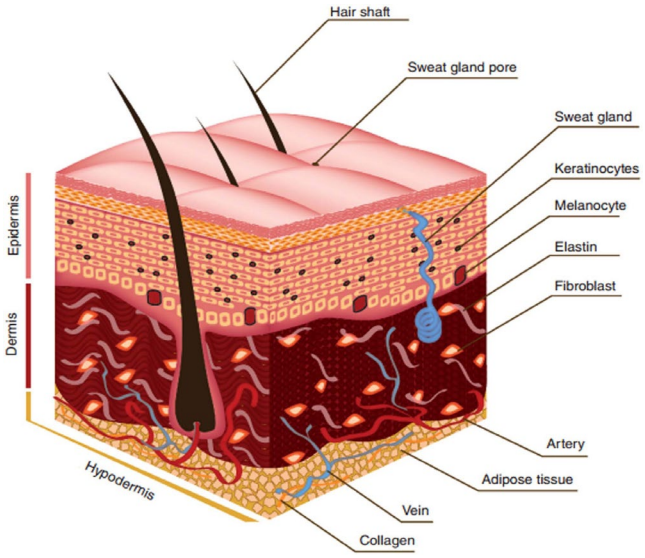

Figure 9. Schematic description of the skin

or synthetic polymers which could promote skin tissue regeneration to certain degree. These substitutes have been used in surgical therapies when autologous flap is not desirable. However, these substitutes have not been successfully used in clinical due to some technological limitations, such as the lack of multi-layer structures, vascularization and innervation[130].

In 2006, Ringeisen *et al.* printed living cells for skin regeneration using a laser-assisted technique[131]. The process employs radiation pressure from the scattering of energetic photons in a laser beam to deposit cell solutions with high concentration, rapid velocity (≥10 m/s) and micrometer resolution. Multiple skin cells were deposited with micron-scale resolution from a transfer layer or reservoir. In 2008, Saunders *et al.* delivered human fibroblasts using a piezoelectric drop-on-demand inkjet printing technique[132]. In 2009, Lee *et al.* used a extrusion-based printing system to fabricate skin substitutes using collagen, fibroblasts and keratinocytes[133]. In 2013,Michael *et al.* further printed skin substitutes using laser-assisted bioprinting techniques and transplanted them to skin wounds of nude mice[134]. It is expected that multiple scale characteristics of a natural skin can be mimicked through the combination of different bioprinting techniques[135].

Recently, skin 3D bioprinting has achieved a significant progress[136]. For example, in 2016 Pourchet *et al.* printed a full-thickness skin substitute containing dermis and epidermis layers[137]. A mixture of gelatin and fibrinogen was used as the "bioink". After 26 days of culture, the 3D printed skin substitute exhibited similar histological characteristics to human skin. Not only the main skin tissues but also the skin appendages, such as sweat glands, has been mimicked[138]. However, the regeneration of sweat glands has not been studied in depth due to the low regenerative ability and unknown induction niches of cellular differentiation. As a follow-up study, Liu *et al.* investigated the cellular niche by tailoring the architecture of a tissue construct *via* cell bioprinting[139]. The change of the geometry and architecture, such as the pore size of the tissue construct, has a strong influence on guiding sweat-gland morphogenesis and function[140]. The studies demonstrate that it is possible to print a bioartificial skin with the sweat-gland regenerative capability.

4. Conclusion

The advent of 3D bioprinting technologies has led to a significant progress in the manufacture of large bioartificial organs, such as the bones, livers, hearts, cartilages and skins, with heterogenic compositions. Various bioprinting techniques have provided a fully automated and advanced platform to deposit multiple cell types and ECM-like biomaterials to simulate the natural organs, a process that is lacking in conventional tissue-engineering approaches. Especially, with the helps of multi-nozzle 3D bioprinters and biocompatible polymers, the divergences between bioartificial organs and native counterparts are smaller and smaller. Nevertheless, there is still a long way to go to make the large bioartificial organs to be functional in clinical trials. It is believed that in the future combined multi-nozzle organ 3D bioprinting technologies will offer an unprecedented versatility and capability in mimicking the natural organs in every aspects, from the structural morphologies, to material compositions, and physiological functions. Further integrations among different sciences and technologies are still necessary to address the kernel issues in large organ 3D bioprinting areas.

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Author Contributions

Xiaohong Wang conceived, designed and wrote the main content; Liu Fan, Chen Liu, Qiuhong Chen, Qiang Ao, Xiaohong Tian, Jun Fan, Weijian Hou and Hao Tong contributed some detailed techniques.

Conflicts of Interest

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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