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REVIEW

3D bioprinting: an emerging technology full of opportunities and challenges

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Abstract
Three-dimensional (3D) printing is a novel promising technology based on 3D imaging and layer-by-layer additive fabrication. It has a profound influence on all aspects of our lives and is playing an increasing important role in many areas including engineering, manufacturing, art, education and medicine. "3D bioprinting" has been put forward with the technical progress in 3D printing and might be a possible way to solve the serious problem of human organ shortage in tissue engineering and regenerative medicine. Many research groups flung them into this area and have already made some gratifying achievements. However, it is a long way to fabricate a live organ. Many elements lead to the limitation of 3D bioprinting. This review introduces the background and development history of 3D bioprinting, compares different approaches of 3D bioprinting and illustrates the key factors of the printing process. Meanwhile, this review also points out existing challenges of 3D bioprinting and has a great prospect. Some points proposed in this review might be served as reference for the research of this field.

Keywords 3D printing · Bioprinting · Printing approaches · Printing factors

Introduction

Organ transplantation is an effective way of medical treatment for the patients who have suffered from severe organ damage. However, the contradiction between the number of organ donated and organs in demand keeps rising because of the rapid growing demand of organs worldwide, even though the number of organs donation is increasing [1]. In the 1980s, the emergence of tissue engineering technology brought the hope of solving such contradiction. Tissue engineering is a new research field that combines biology, material science, engineering, surgery and molecular technology, the goal of which is to solve the current predicament of organ shortage by fabricating tissue substitutes with biological functions [2–4].

In conventional tissue engineering approaches, porous scaffolds were first produced by salt leaching, electrospinning or other foaming techniques, then seeded with cells and grew with growth factors to form three-dimensional structures, and then the cells were guided to form various desired tissues [5,6]. Nowadays, a variety of tissue engineering technologies based on scaffold structures have been developed rapidly, but there still remained some defects to be solved: (1) It costs too long to produce tissue organ which delays the treatment; (2) it is unable to carry out multi-cell implantation, and the cells cannot achieve distribution with high density and precision spatial position; (3) it is hard to achieve the oriented growth of blood vessels and still has problems on the nutrition supply for large organs [3,7]. New fabrication method to achieve fast organ production with precision spatial position of multi-cells/materials and blood vessel orientation is really needed.

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3D printing technology, also known as the rapid prototyping, is a new technology invented in the 1980s, which the digital model is scanned into slices through computer-aided manufacturing technology and then constructed the 3D geometry by means of layer-by-layer printing [8,9]. The application of this technology in life science and medicine has gradually become a multidisciplinary field called 3D bioprinting, which has been used in customized model, human permanent implants, biomimetic scaffolds, drug testing model and controlled drug releasing; this technology plays a more and more important role in the modern biomedical applications [10–12]. Different from ordinary 3D printing technology, the materials in 3D bioprinting are mainly cells, biological materials and growth factors, etc., and the 3D bioprinting technique applies computer-aided design software to build 3D models of tissues and organs, transmits the generated information to the control center and controls the speed and position of printing nozzles for precisely depositing cells and materials. It can accurately control the scaffold morphology and internal stent materials, construct artificial tissue with highly similar structure of human body, solve the major problem of accuracy and precision of scaffold and cell implantation [13]. The 3D bioprinting technology has incomparable advantages especially for heterogeneity tissue construction with multi-cells/materials.

In the past several years, 3D bioprinting has made new process in many aspects, like printing models, materials, printing methods, but there is still a long way to truly substitute the human organs with this technology. In order to promote the development of 3D bioprinting, the suitable way is to find out the most important factors in the printing process, realize the challenge of fabricating real organs and try to take measures to solve them. In this review, we will present some of the cutting edge 3D bioprinting applications around the world, then introduce mainstream printing approaches, analyze the key factors during the printing process, point out challenges and difficulties and finally make the future prospect of this technology (Figs. 1, 2, 3).

Applications of 3D bioprinting

Nowadays, 3D bioprinting is successfully used in many areas in tissue engineering and regenerative medicine such as hard tissue, soft tissue and disease model construction. In this review we take cartilage, skin and tumor as examples to summarize the current updates in constructing the above tissues and organs with 3D bioprinting.

Cartilage printing

Cartilage is an elastic connective tissue that supports and protects the human body. Cartilages are distributed in various parts of human body, as a support structure in the ear, nose, bronchus and other parts, and play the buffer and protection role of the bone in the joint. Cartilage damage cannot be repaired by itself, in that case the damaged cartilage must be replaced with substitutes. The use of 3D bioprinting technology has a great application prospect in the cartilage tissue repairment, and many previous researches had shown the potential of using this novel technology in this area. Martínez et al. [14] used a kind of bioink composed of nanofibrillated cellulose and alginate (NCF-A) laden with human nasal chondrocytes (hNC) to print out the structure similar to the ear. And it produced glycosaminoglycans (GAG), collagen type II (COL2A1), etc., in gene expression aspects. Lee et al. [15] used polyethylene glycol (PEG) as the sacrificial layer, laden chondrocytes and adipocytes in the hydrogels, respectively, added polycaprolactone (PCL) as the composite material, adopted six needle 3D bioprinter, to achieve multi-cell tissue printing for the regeneration of both the auricular cartilage and fat tissue. Gao et al. [16] compared using PEG and polyethylene glycol–gelatin methacrylate (PEG-GelMA) as the printing material, respectively, to study the osteogenic and chondrogenic differentiation capacity of human mesenchymal stem cells (MSCs). The results show that the addition of appropriate proportion of GelMA not only improves the mechanical properties of the material, but also can promote the differentiation capacity. On recently researches, nanocellulose-based bioink was printed with chondrocytes to form cartilage tissues, resulting high cell viability, morphology and matrix deposition [17,18]. One of the major challenges in bioprinting cartilage is the weak mechanical properties. Cui et al. [19] removed a part of the animal femur, made a cylindrical gap in the cartilage part, printed polyethylene glycol dimethacrylate (PEGDMA) and human chondrocytes in the gap. Chondrocytes were distributed evenly in the 3D structure by using simultaneous photopolymerization, and the compressive modulus of printed PEGDMA was 395.73 ± 80.40 kPa, which was still 20% less than native human articular cartilage. The stabilization of the implanted cartilage tissues in the joint and the integration between the implants to surrounding native tissue make big challenges to cartilage recovery, promotes researches focus on the structures, bioinks of cartilage printing, but maybe mostly rely on the tissue self-assembly approaches [20].

Skin printing

Skin is the largest organ in the human body, accounting for about 15% of the total body weight in adults [21]. It plays a significance role of protection, perception and regulation functions [22] and keeps the body away from possible invasion and maintains the body circulation. Many diseases and injuries lead to skin loss, thermal trauma, scratches and dia-

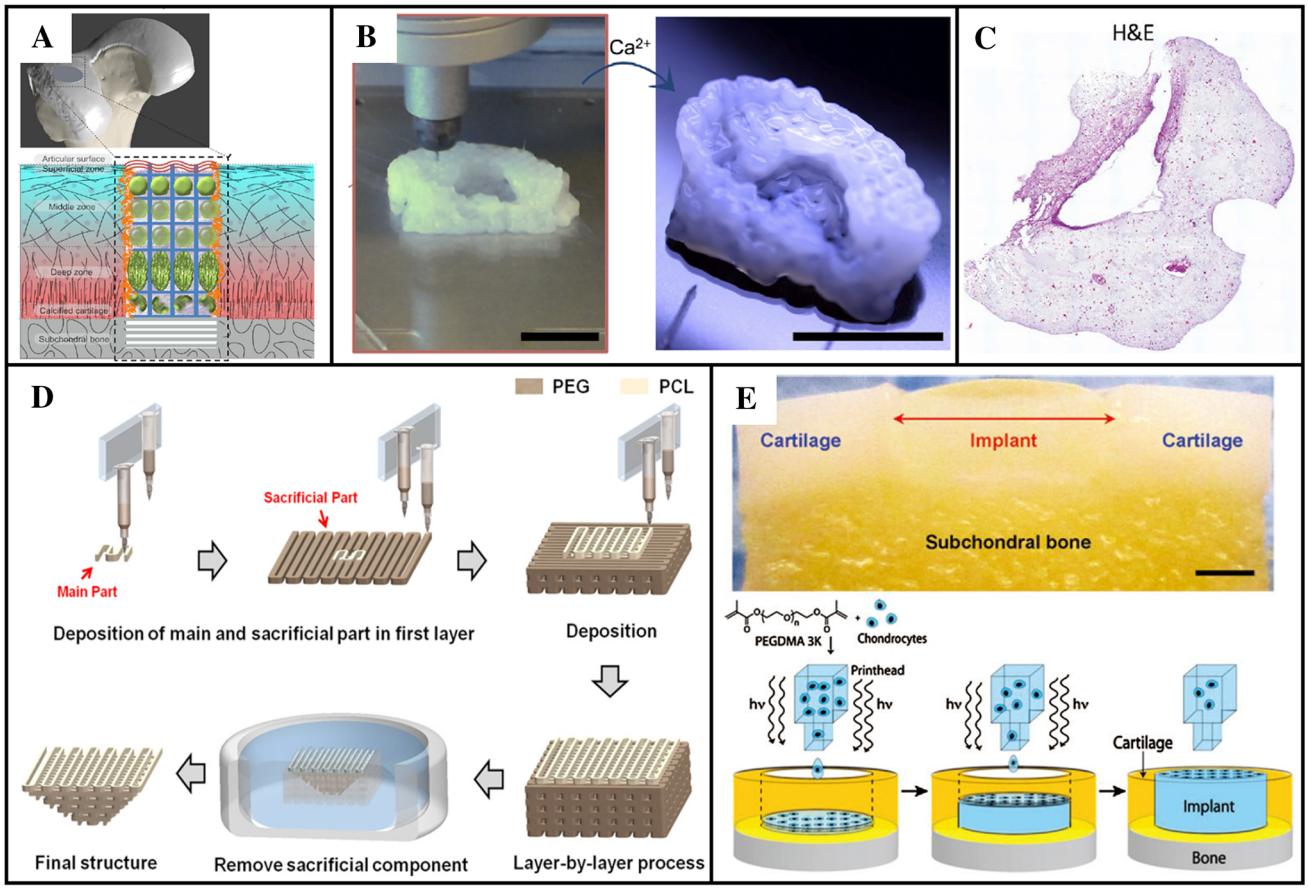

Fig. 1 Cartilage printing. **a** Schematic illustrating cartilage structure [20]. **b** Printed ear cartilage [14]. **c** Hematoxylin and eosin (H&E) for extracellular matrix of printed chondrocytes after 5 weeks [18]. **d** Schematics illustrating printing process of porous cartilage tissue using sacrificial layer technology [15]. **e** Light microscopy image of PEG cartilage plug printed in the osteochondral defect and its printing process [19]

betic foot ulcer cause full-thickness skin defects for millions of people each year and cost billion dollars in health care [23,24]. With cosmetic products tested on animals forbidden, functional artificial skins are extremely needed [25]. Skin is a distinct layer structure with epidermis, dermis and hypodermis [26], and researchers construct skin with layer-by-layer printing methods. To acquire the functional skin models, inkjet printing was firstly proved to construct dermal/epidermal like scaffold with keratinocytes and fibroblasts [27], after cultured at air–liquid interface (ALI), the dermal and epidermal layers appeared [28]. Furthermore, melanocytes were printed between the two layers to form ephelide in biomimetic skin [29] and offer the capability of producing color skin of different races. The amniotic fluid-derived stem(AFS) was accurately printed in the mice dorsum detect area [30], healing wounds by self-differentiation of stem cells. The closure rate was obviously increased compared to the control group and demonstrates a promising approach for effective wound healing. Extrusion method for skin printing constructs thick and viable dermis in a matter of minutes [31], and combined with inkjet, the skin model with functional transwell system was fabricated in a single-step process [32]. Besides the structure of two layers, skin appendages printing is under way. Vessel channels were patterned at the bottom of skin, with the tissue engineering method to form dermis and epidermis [33]. In this research, micropatterned vascular networks were established, providing oxygen and nutrition for skin which thus promote the vasculogenesis. A suitable microenvironment for sweat gland regeneration was established by 3D bioprinting technology and primary sweat gland structure formed [34]. With further study, the accurate and organized 3D ECM with controlled pore construction guides the glandular morphogenesis [35]. However, the sweat gland printing only mixed several materials together as a kind of bioink, induced the sweat gland rely on cell self-differentiation and cost long time for generation. The further research of skin appendages should focus more on the structure, make 3D bioprinting work to its advantages and precisely control the target tissues.

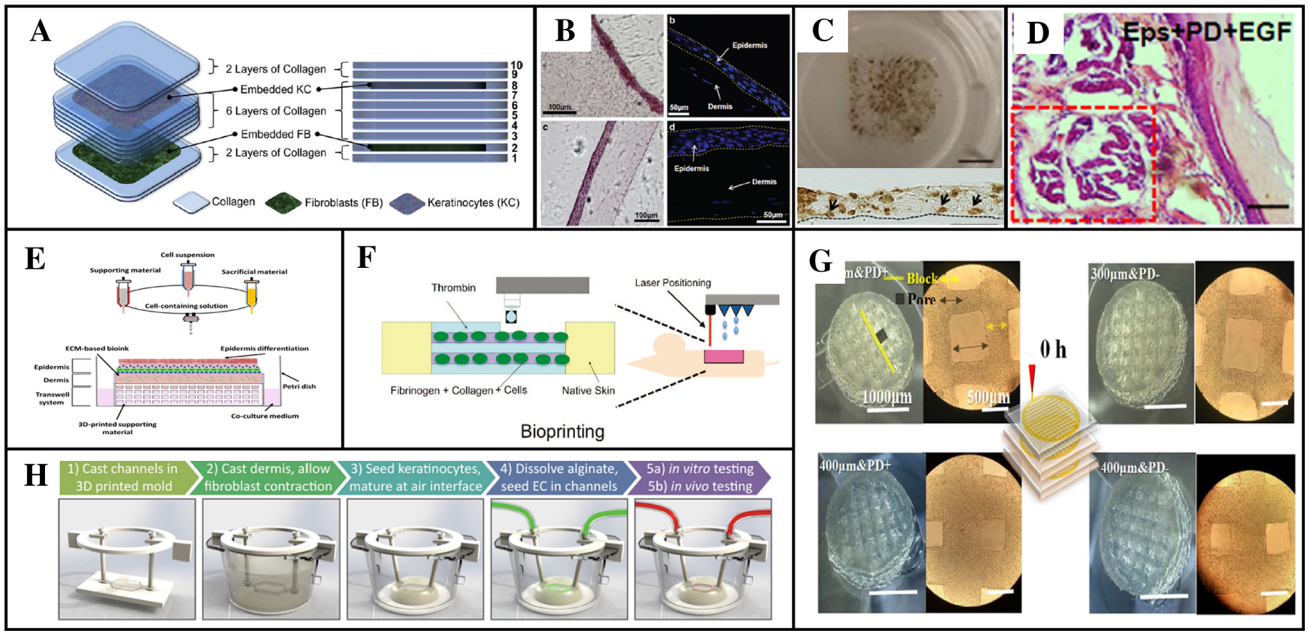

Fig. 2 Skin printing. **a** Schematic of inkjet printing with skin cells and collagen [27]. **b** Epidermis and dermis structure after ALI cultured [28]. **c** Printing skin with melanocytes [29]. **d** Histology of wound healing with sweat glands like structure [34]. **e** Schematic diagram describing approach of printing skin–transwell system [32]. **f** Healing mice dorsal wounds with inkjet printing [30]. **g** Fabricate pore scaffolds to guide sweat glands regeneration [35]. **h** Schematic description of the vascularized skin models construct process [33]

Tumor printing

As a versatile platform, 3D bioprinting has become an emerging biofabrication technique that enables construction of in vitro 3D tumor microenvironment models with high resolution and throughput. In comparison with conventional tissue engineering methods, 3D bioprinting has enhanced the biomimetic properties and functionality of tumor models by providing accurate and proper composition of the complex tumor environment along with well-organized spatial distribution of tumor cells and extracellular matrix (ECM) components. In addition, 3D bioprinting has the ability of vascularization at great fidelity. As a result, bioprinted tumor models could successfully recapitulate the types and stages of the in vivo tumor progression of human patients and, therefore, could be used for fundamental biological studies and more efficient anticancer drug screening to eventually achieve personalized anticancer therapies.

Several applications of bioprinting in establishment of biomimetic tumor models have been illuminated to date. Seung-Schik Yoo et al. built a glioblastoma (GBM)–vascular niche combining the sacrificial vascularization and multicellular spheroids. In this model, patient-derived GBM cell spheroid was located beside the perfusable vessel [36]. The influence of microenvironmental factors has been investigated for better control on the cell behavior. Tao Xu et al. used a custom-made coaxial extrusion 3D bioprinting system to fabricate self-assembled multi-cellular heterogeneous brain tumor fibers for studying tumor–stromal interactions. Results showed that tumor–stroma cells interacted with each other and fused [37]. Wei Sun et al. encapsulated HeLa cells in a composite bioink and bioprinted a 3D cervical cancer model for drug testing. It was revealed that the bioprinted 3D model was more realistic than the conventional 2D cultures [38]. Utkan Demirci et al. constructed a coculture model with OVCAR-5 ovarian cancer cells and normal fibroblasts in Matrigel [39]. The 3D droplet-based tumor model enabled a better control of the spatial distribution of the cells and the cell density in the tumor microenvironment, providing a more reproducible cell patterning. Kristopher A. Kilian et al. created a coextrusion bioprinting model to study the interactions between MDA-MB 231 breast cancer cells and macrophages. The model enabled the analysis of the interactions between tumor cells and the surrounding stromal cells in the vicinity [40]. Lijie Grace Zhang et al. developed a biomimetic bone matrix where breast cancer (BrCa) cells were introduced into the stromal cell-laden bioprinted matrices to investigate the interaction between BrCa cells and bone stromal cells [41].

Major technologies for 3D bioprinting

Bioprinting technology mainly includes the following three types: laser printing, inkjet printing and extrusion printing [42]. Based on the CAD model of tissues and organs, cells

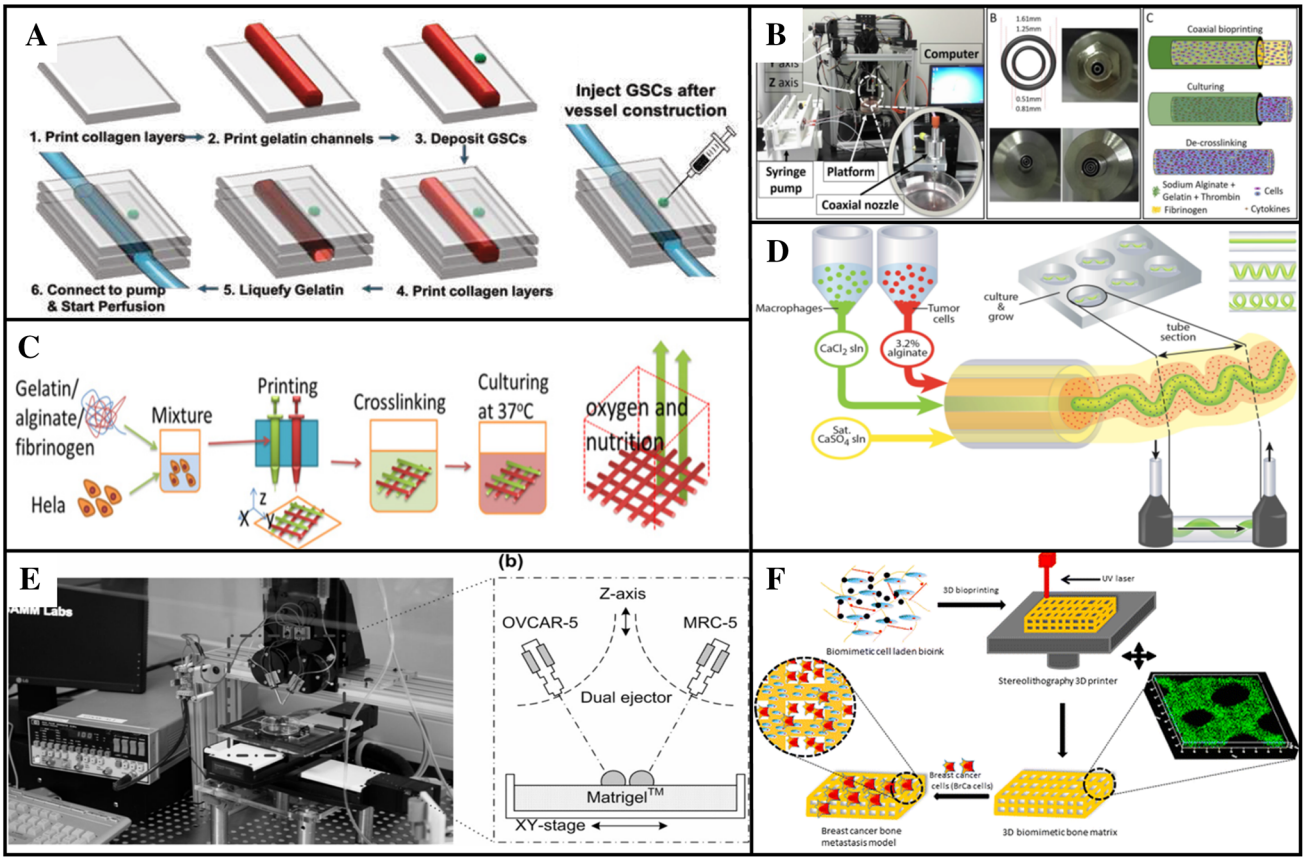

Fig. 3 Tumor bioprinting. **a** Schematic of glioblastoma–vascular niche [36]; **b** coaxial extrusion 3D bioprinting system fabricating self-assembled multi-cellular heterogeneous brain tumor fibers [37]; **c** 3D cervical cancer model with HeLa cells in a composite bioink for drug testing [38]; **d** coextrusion bioprinting model with MDA-MB 231 breast cancer cells and macrophages [40]; **e** coculture model with OVCAR-5 ovarian cancer cells and normal fibroblasts in Matrigel [39]; **f** schematic of biomimetic bone matrix with breast cancer (BrCa) cells introduced into the stromal cell-laden matrices [41]

and biomaterials are combined to make what we need through printing step by step. Differing from the traditional composite manufacturing way of "ells and scaffolds", it provides accurate spatial positioning for the cells. A variety of living cells or biological materials can be printed simultaneously, which includes specific cells of particular organs and blood vessels (such as muscle cells and endothelial cells) [43], and also it can create living organs with functional vascularized network. Although the technology is still in the infancy stage at present, it shows great potential in the field of tissue engineering and is expected to ease the existing shortage of substitution organs for transplantation (Table 1).

Laser-assisted printing

The technology of laser printing has been used to print cells. It mainly applies optical tweezers effect and thermal shock effect to trace laser to deposit droplets which contain cells [44]. In 1999, Odde et al. [48] used laser system to cope with 2D cellular structures. The principle was to use laser deposition reaction to deposit cells; when the laser beam acted on cells or droplets which contain cells, it would produce an effect of two components in different directions to the droplets, so that the cells would move in the direction of parallel and vertical beam. Through the control of the laser beam, cells can be controlled accurately in the whole process from separating, moving to arriving at the base plate. This technology is called laser-guided direct writing (LGDW). Nahmia et al. [47] realized the configuration of liver cells in collagen and artificial basement membrane through using LGDW. In this study, they finally used cells and hydrogels to produce 3D structure with three layer structure successfully, and in the process of reprocessing, cells kept good ability of survival and differentiation. Figure 4a shows the schematic of laser-assisted printing.

The major advantage of laser-assisted bioprinting is high resolution of the system. It can precisely control the spatial location of a single droplet of cell to ensure the accurate arrangement of living cells. As a kind of non-contact technology, laser-assisted bioprinting can also print a variety of

Table 1 Features of different bioprinting methods

Print methods	Bioinks	Resolution	Cell viability	Cell density	Print speed	Target tissue	References
Laser-assisted printing	Fibrinogen, collagen, GelMA	1–50 μm	> 97%	10^8 cells/ml	100–1600 mm/s	Skin, vessel	[44–49]
Inkjet printing	Collagen, poly(ethylene glycol) dimethacrylate (PEGDMA), fibrinogen, alginate, GelMA	50–500 μm	85–98%	$<5 \times 10^6$ cells/ml	1000–5000 droplets/s	Skin, cartilage, bone, tumor, liver	[19,27–29,32,50,51]
Extrusion printing	Gelatin, polycaprolactone (PCL), polyethylene glycol (PEG), alginate, hyaluronic acid (HA), polyamide(PA), polydimethylsiloxane (PDMS) dECM, nanocellulose	> 50 μm	80–96%	Cell spheroid	5–20 mm/s	Skin, cartilage, vessel, bone, muscle, tumor, heart	[14,15,17,34,52–59]

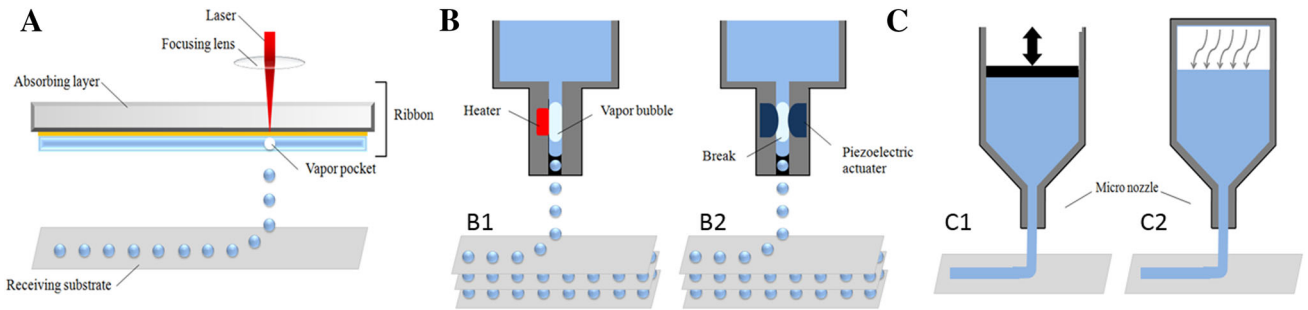

Fig. 4 Schematic of major technologies of 3D bioprinting. **a** Laser-assisted printing; **b** inkjet printing; **b1** thermal inkjet printing; **b2** piezoelectric inkjet printing; **c** extrusion printing **c1** piston extrusion printing; **c2** pneumatic extrusion printing

biological materials and cells in different culture medium to prevent cell pollution and medium damage. In addition, the technology also has advantages of high cell survival rate, small volume of droplet [46,60].

Although the LGDW technology can achieve high-resolution single-cell printing, still there are some limitations of current laser-assisted bioprinting: (1) low printing efficiency and relatively long time of manufacturing process. The droplet generation velocity is only drops per second, which cannot reach the requirement of organization; (2) the thermal shock of laser might easily cause the deformation or even damage to the cells; (3) laser printing has its limitations in the third dimension of printing. In order to realize complex printing of cell-contained mimetic structures, it is necessary to extend its printing function in the third dimension [1]. In addition, in order to further improve the resolution and production capacity of laser bioprinting, it is necessary to optimize the characteristics of laser pulse parameters (such as pulse cycle, pulse length and pulse beam diameter, energy), solution (including solution viscosity, thickness, surface tension) and base plate [61].

Inkjet printing

Since the early twentieth century, inkjet bioprinting technology has experienced rapidly development. It is the earliest use of biological printing technology. It adopts non-contact printing technology using ink (cells or biological materials)

to realize organ fabrication based on the digital models of tissues and organs in computer [62]. The technology could be mainly divided into two types: thermal inkjet printing (Fig. 4b1) and piezoelectric inkjet printing (Fig. 4b2) [63].

Thermal inkjet printing technology mainly uses heating element to spray droplets. The element is used to heat the adjacent area of ink rapidly, so that the ink in the pressure cavity will be gasified into bubbles [64]. The pressure generated from gasification makes a part of the droplet overcome surface tension and be squeezed out of the extrusion nozzle; when voltage is released, bioink cools quickly and returns back into the pressure chamber. The droplets can be squeezed continuously by applying and releasing voltage repeatedly. Piezoelectric inkjet printing technology mainly uses piezoelectric materials to spray droplets. When voltage is applied on both ends of the piezoelectric element, piezoelectric element bends and droplet near the nozzle is squeezed out. When voltage is released, the piezoelectric element restores back to its original shape [65]. In this way it can achieve continuous extrusion drops through applying and releasing voltage repeatedly. Cui et al. [19] used inkjet printing technology to repair articular cartilage of human body, which was proved to be potential to lead tissue to regenerate efficiently. The system was by means of controlling the process parameters such as cell concentration, volume and precision of droplet, nozzle diameter and average diameter of printing cells to print cells and biological materials [66]. Weiss et al. [67,68] developed an inkjet printing platform with multi-nozzles to manufacture composite structures. A variety of growth factors, such as fibrinogen and thrombin, together with cells were printed precisely into cell skull defect of mice. They showed the feasibility of *in-situ* printing; however, due to the complexity of this process, it was not suitable for practical use.

Inkjet printing technology has been applied earlier so it is relatively mature, and its main advantages include: (1) Similar to color printing, inkjet printing can integrate multiple nozzles to synchronously print cells, growth factors, biological materials together and is capable of building heterogeneous tissues and organs [69]; (2) inkjet printing is a non-contact way of biofabrication. Nozzles and the culture medium are separate, so possible cross-contamination in the printing process can be prevented [70]. It can be printed on solid, hydrogels and liquid interfaces. And there is no additional requirement for printing graphic smoothness, which is advantageous to the *in situ* print; (3) inkjet printing is in high speed and high efficiency, which is beneficial to solve the organ printing-related problems such as longer production time and biological activity decline, and is suitable for large parts manufacturing; (4) the droplet volume is small, similar to a single body cell size, so that precise operation can be realized to the individual cell [71].

Although there are many research achievements with inkjet printing technology, still there are some limitations: (1) Because the nozzle diameter is too small, the cells are more likely to precipitate and accumulate, which limits the printing density ($< 5 \times 10^6$ cells/ml) [1]; (2) during the thermal printing process the nozzle is heated to a very high temperature, so it would be harmful to cells, and at the same time the existence of shear stress would also reduce the cellular activities; (3) the fusion between droplets is not easy, and the shape of the droplets cannot be accurately controlled. The structural integrity of printing is also a problem need to be solved when using inkjet printing.

Extrusion printing

Extrusion printing technology enables to fabricate living cells and materials in continuous lines [72]. It combines the fluid distribution system and the three-axis robot automatic control system to print cells. In the process of printing, biomaterials are extruded through the pressure auxiliary system [either piston (Fig. 4c1) or pneumatic (Fig. 4c2)] with robotic control. In the deposition process, the cells are mixed with materials solution, so they can be deposited to form an ideal 3D structure accurately in the form of lines [53,73]. Yan et al. [74] used 3D bioprinting system to deposit different types of cells and various biocompatibility hydrogels. They used liver cells and fatty liver cells (adipose derived stromal cells, ADSCs) to create artificial liver with gelatin/chitosan hydrogel. Khalil et al. [55] developed a multi-nozzle printing system, which can deposit cells together with a variety of biomaterials at the same time. They studied the rheology and cellular activity to study the mechanical shear stress that lead to cell damage in the process of printing [75]. Their results showed that the cell activity is affected by flow velocity, concentration, deposition pressure and nozzle geometry. Their research can be used to guide the future study of sedimentary system and optimization.

Since extrusion-based systems can realize continuous line traces deposition, it reaches well structural integrity [76]. The main limitations of this technique are shear stress effect and narrow material selection. The shear stress induced by the extrusion process will cause cells to deform and damage. When the cell density is too high, the shear stress in nozzle wall will cause a decline in number of living cells; however, through the optimization of process parameters such as the concentration of the biomaterials, nozzle pressure (ideally it should be as small as possible), nozzle diameter and cell density, this problem can be partially alleviated. The material choice is limited, because the material needs to be able to encapsulate cells through hydrogels. Limited range of material selection, low resolution and accuracy limit the application of extrusion system. In addition, as for biological suspension, the viscosity needs to be high enough to overcome the cell deformation caused by surface tension and it should be guided to form a straight line. On the other hand,

the high viscosity will lead to clog in the nozzle, so it is necessary to optimize the viscosity according to the nozzle diameter.

Key factors

3D bioprinting is different from the original printing, which needs to control more complex conditions to avoid damage to the cells [77]. There are many factors influencing the fabrication process, and this review lists several key factors as follows:

Material properties

3D bioprinting adopts biomaterials to construct extracellular matrix, which provides the environment for cell adhesion, growth, proliferation, migration and differentiation [78]. The *in vitro* microenvironment should be similar to the *in vivo* body environment, supporting the cell metabolism. Biomaterials should have excellent biocompatibility, good mechanical strength and outstanding forming performance at the same time, to form a biological scaffold with sufficient supporting ability and the scaffold can provide space for cells survival. Materials should have the ability to switch between liquid and solid: It needs to be at liquid or molten condition which is beneficial to deposition during the printing, but immediately transform to solid, in that case the following materials can be successively deposited and complete the model layer by layer. Generally, this process requires cross-linking methods for assistance [79–81]. Meanwhile, materials need to have the appropriate degradation ability, which means the material can degrade in the wanted time, and the byproduct of the degradation won't have any impact on the cells and other parts of human body, or as known as cytotoxicity free [82].

Scaffolds structure

3D bioprinting is a method of constructing *in vitro* human organs, the tissue and organs fabricated ought to achieve a high degree of similarity not only in shape but also in functionality with the human body, which makes great significance of the biological scaffolds [83]. First of all, the scaffolds structure must be stable enough to form a large tissue, avoiding any collapse and destruction during the printing process. Secondly, the structure should have certain porosity for cell growth [84], since there is no ability to really establish the microvascular distribution with good substance exchange capacity at present. Especially, when the distance between cells and nutrients is larger than 200 micron, the normal supply for cells would be unable to complete [85], which can be solved by suitable structure design. Last but not the least, as for the construction of complex organs, a simple structure may not satisfy the multi-cellular demand. For example, when it needs to fabricate the inverted structure, there must be a sacrificial layer below to support the whole structure [56,86].

Printing precision

As for 3D bioprinting, the best state is to control the single-cell deposition, so that the cells can be located accurately to construct an ideal organizational structure. Especially for complex multi-cellular organs, single-cell control can simulate the structure of the human body to the most extent and be exactly the same with human organs when there is enough cell sources. In that case, the interaction of cells can be controlled artificially. Existing bioprinting technologies cannot achieve the single-cell control [87–90], which means improve the accuracy of the printer and coupled with a reasonable control algorithm is an important way to enhance the function of the fabricated tissues and organs.

Environmental control

External factors such as temperature and humidity are also important factors to maintain the activity of tissues and organs. The temperature can directly affect the activity of the cellular protein in the scaffolds, which has a great influence on the tissue function. The suitable humidity has important effect on the cell growth and proliferation. Some groups tested post-printing survival rate of cells and found that the temperature between 29 and 31 degrees centigrade is suitable for biological materials like collagen, and the cell viability was the highest when the humidity levels remained at 65–85% [91]. However, different cells have different requirements for temperature and humidity, and there is still a long way to analyze the influence of different environmental factors with various materials.

Sterile condition

As the requirements of precision improvement, the printing time increases so that cells may overexposure to the outside environment, which means the contamination possibility is high . The aseptic measures should be taken into account during this process to protect the cells. Aseptic processing needs to carry out gradually according to the status of machine and printing process, to ensure the cell viability of organs after printing, so that the printed organs can survive a long time with their functionalities [60].

Challenges

The main challenges of 3D bioprinting are the suitable material and nutrient supply to the cells, which has stunted the process of this technology for several years [92–96].

Material selection

Various types of biomaterials used in 3D bioprinting can be broadly divided into two groups based on the source of production, namely natural polymers like collagen, chitosan, hyaluronic and synthetic polymers such as polylactic acid (PLA), polyglycolic acid (PGA), poly(lactic-co-glycolic acid) (PLGA) [97]. Natural biopolymers have good biocompatibility, but usually perform poorer at mechanical properties, which makes it achieve the required formability as a single printed material. By contrast, synthetic biopolymers generally have good formability with poor biocompatibility. Researchers are focusing on building blending system between different materials since the single material always can't meet the manufacturing needs [80], which has both natural and synthetic materials constituents, in that way combining the advantages of both materials and overcoming the limitations to achieve the demand material properties. Meanwhile, another popular method is to control the cross-linking parameters, such as the cross-linking reactions between hydrogels to improve the forming property [98], which makes it possible to achieve good biocompatibility and suitable formability. There is also light curing, changing PH value, adding calcium chloride solution, adding nanomaterials and other approaches to cross-link the hydrogels [87,99], which make a difference to the performance of biomaterials. Seeking suitable cross-linking methods is the way to achieve good formability of fluid biomaterial and to meet the demand of shape. However, all the methods mentioned above have a certain impact on cell survival. As consequence, finding a feasible material and its cross-linking mode is still the main goal of academic society in this area now.

Nutrient supply

Nowadays, a difficult problem in the construction of *in vitro* large-size organs lies in the inability to achieve effective microvasculature [7]; thus, it is difficult to supply nutrition to the cells in the interior section of the constructed organ. One current method is to construct some channels inside the biofabricated organs and perfuse nutrients to simulate vasculature; then endothelial cells, smooth muscle cells or fibroblast cells are injected to form vascular structures [10,100–102]. But there is still a long way to achieve real vascularized structures. Some researchers also found that printing large vessels in certain areas, will induce microvasculatures to generate asides [103], though which can't meet the construction of all organs. How to build a microvascular network still remains a big challenge in the field.

Conclusions and outlook

The*in vitro* 3D bioprinting technology has unparalleled advantages in constructing human body defect prosthesis, organ transplantation and drug testing, etc [104,105]. Although this field is still in its early stage with many challenges, considerable amount of results have generated great practical value, such as the 3D bioprinted bones [106] which are under human clinical trials and have a potential in future medical applications [107]. There are many elements during the fabrication. If we take all the key factors into account and take enough experiments, the printing will avoid a lot of mistakes and achieve better results. 3D bioprinting is a multidisciplinary technology that requires the common knowledge of engineering, biology, material, information as well as medicine. It is experiencing a rapid development along with the progress of above fields. With the joint efforts of researches in generations, 3D biofabrication will become one of the promising future mainstream technologies in medicine and bioengineering.

Many countries currently regard 3D bioprinting as key development project, and a large number of universities and companies are carrying out in-depth study in this area [108], which is due to the tremendous potential of 3D bioprinting. 3D bioprinting not only benefits ordinary people, but also makes a great difference to our countries, such as *in-situ* printing. It is obvious that the 3D bioprinting will have a bright future, and is destined to benefit our society.

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