



3D Printing: Advancement in Biogenerative Engineering to Combat Shortage of Organs and Bioapplicable Materials

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Abstract

Organ or cell transplantation is medically evaluated for end-stage failure saving or extending the lives of thousands of patients who are suffering from organ failure disorders. The unavailability of adequate organs for transplantation to meet the existing demand is a major challenge in the medical field. This led to day-day-increase in the number of patients on transplant waiting lists as well as in the number of patients dying while on the queue. Recently, technological advancements in the field of biogenerative engineering have the potential to regenerate tissues and, in some cases, create new tissues and organs. In this context, major advances and innovations are being made in the fields of tissue engineering and regenerative medicine which have a huge impact on the scientific community is three-dimensional bioprinting (3D bioprinting) of tissues and organs. Besides this, the decellularization of organs and using this as a scaffold for generating new organs through the recellularization process shows promising results. This review discussed about current approaches for tissue and organ engineering including methods of scaffold designing, recent advances in 3D bioprinting, organs regenerated successfully using 3D printing, and extended application of 3D bioprinting technique in the field of medicine. Besides this, information about commercially available 3D printers has also been included in this article.

Lay Summary

Today's need for organs for the transplantation process in order to save a patient's life or to enhance the survival rate of diseased one is the prime concern among the scientific community. Recent, advances in the field of biogenerative engineering have the potential to regenerate tissues and create organs compatible with the patient's body. In this context, major advances and innovations are being made in the fields of tissue engineering and regenerative medicine which have a huge impact on the scientific community is three-dimensional bioprinting (3D bioprinting) of tissues and organs. Besides this, the decellularization of organs and using this as a scaffold for generating new organs through the recellularization process shows promising results. This review dealt with the current approaches for tissue and organ engineering including methods of scaffold designing, recent advances in 3D bioprinting, organs regenerated successfully using 3D printing, and extended application of 3D bioprinting technique in the field of medicine. Furthermore, information about commercially available 3D printers has also been included in this article.

Keywords 3D bioprinting · Organ transplantation · Organ donor · Decellularization · Scaffold designing · Drug efficacy testing

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Introduction

In this world, half of a million patients are estimated on the waiting list for an organ transplant, and due to the inaccessibility of organs, mortality rates increase due to the failure of a vital organ. A deceased donor or a living donor can donate 25 different organs or tissues which can save as many lives as possible [1–3]. Organs such as the kidney, liver, pancreas, lungs and heart, eyes, skin, bone, bone marrow, nerves, brain, heart valves, eardrum, ear bones, and blood can be donated [4–7]. The organ transplantation process seems too easy but identifying a perfect match of the organ is very difficult. Apart from the ethical approval-related hurdles and lack of awareness, organ donation comes with a bag of challenges which include red-tapism and king-sized paperwork. Another challenge is to convince family members for organ donation of their loved ones who are on life support due to deceased or brain-dead situations. Besides this, there are very few hospitals that have equipment for organ preservation and transplantation which further limits the usage of organs of brain-dead patients [8, 9]. Furthermore, the cost of organ transplantation and medication followed by surgery cannot be afforded by people belonging to low socioeconomic groups or middle-income countries.

Recent advances in the field of tissue engineering and regenerative medicine not only open new avenues to replace and regenerate tissues and organs but also have the potential to correct some birth defects via lab-grown organs and tissue, bioartificial organs, or using xenogeneic organs. Since cells used in regenerative medicine and tissue engineering are taken from the same patient (autologous), therefore the rejection of transplanted tissue/organ via patients' immune system can be avoided, while cells/tissues from another individual (allogeneic) can elicit an immune reaction, which limits their application. However, the immune response can be suppressed by immunosuppressant drugs, but these medicines have several unavoidable side effects. The alternative types of cells from other species known as xenogeneic cells can also be used in regenerative medicine [10, 11].

Tissue engineering is the process of creating specific organs by constructing a scaffold out of biomaterials, stem cells, and specific growth factors, which can be implemented using 3D bioprinting, an additive manufacturing technique. This technology is used to create 3D structures by adding material on a layer-by-layer process. This is an industrial fabrication technology that offers rapid and mass production of components [12, 13].

In 3D printing technology tissue or organ, drafting is done by computer-aided software using the specific program which specifically provides instruction to a machine for the generation of 3D scaffolds [13]. However, one of the utmost favorable scaffold designing method is decellularization of organ retaining extracellular matrix (ECM) then recellularization by

autologous or stem cells which led to growth and development of the more mature fully personalized organ. The decellularization method removes immune cells, maintaining ECM, and offers equally physical and mechanical microenvironment required by cells to endure and multiply [14–19]. Recently, Alperen Abaci et al. have shown applications of both 3D bioprinting and decellularization technology in developing the appropriate bioink formulation to fabricate 3D constructs and developing the tissue or organ decellularization methods [16]. Similarly, Kabirian et al. have elaborated the applications of dECM-based bioinks for 3D printing technology [17]. The recent reviews presented the designing dECM-based bioinks [18, 19] and their application in 3D printing techniques with limited focus. The present review dealt with the advances in the fields of 3D bioprinting which includes the 3D bioprinters, their types and specification, process of decellularization, bioink formulation, biomaterial selection, and clinically synthesized and developed 3D organs and tissue. Besides, this current approach for 3D printing technology along with information about commercially available 3D printers has also been discussed.

Current Approach Towards the Tissue and Organ Engineering

The end-stage organ failure demands the functionally active organ and tissues which are different in structure and function; however, the engineered material could have a similar composition and morphology as the targeted ones. In this context, for developing a functionally transplantable bioartificial organ, certain factors such as type of organ scaffold and method of its sterilization, designing and maintaining of the vascular network of the scaffold, assessment of immunological barriers, type of cells/tissue to regenerate and cells for infusion, functional aspects of the regenerated organ, and route of cell delivery are crucial factors to be considered. Apart from this, assessment of long-term cell survival and engraftment and need for induction factors must be taken into account cautiously [20, 21].

Scaffold Designing Approaches

Scaffolds are used to restore, replace, or regenerate the damaged tissue or organ. Scaffold designing was introduced in the mid-1980s in the field of tissue engineering and since then it continues to grow and evolve as a multidisciplinary field. Scaffolds are mainly made from biomaterials such as polymers, biocomposites, and bioceramics [22, 23] which provide the framework for cell attachment and tissue development, cell proliferation, host integration, and vascularization [21]. The successful strategy for designing the scaffolds which includes all features such as biological, mechanical, and

architectural [24, 25] is described in Fig. 1. The scaffolds, which were created using computer-aided design software, must have biomedically relevant design features. As the scaffold provides supporting frame to cells for proliferation and differentiation, therefore, it should be biocompatible and does not provoke immune responses resulting in minimal cell cytotoxicity.

The scaffolds provide mechanical strength and shape to bioengineered tissue. Therefore, biomaterials used for scaffold fabrication should possess good mechanical strength and should be compatible with the shape of the organ to be replaced. Furthermore, the design for the scaffold should be capable of providing enough space for the vascular networks and regeneration of the new tissues. The structure of scaffold should mimic the properties of original body organ and should allow efficient transport of metabolites and nutrients to growing cells/tissues. At the time of implantation, the biomaterial shall be biodegradable at an equal rate to that of a matrix manufacturing by developing tissue. The biomaterial bioink chosen for the fabrication of scaffolds must be biologically active and compatible with the host system; thereby, the engineered tissue actively facilitates and regulates its function. The scaffold may regulate signaling pathways such as the formation of growth factors to speed up the process of regeneration. There are numerous 3D bioprinting approaches, tools, and biomaterials available to fabricate porous scaffolds with organized porosity and pore sizes such as stereolithography and robocasting [26, 27].

Scaffolds are biodegradable, mechanically robust structures that are effective at transporting metabolites and nutrients. As explained in the previous section, there are various technological approaches to fabricate the scaffolds. The two of them are decellularization and 3D bioprinting. Decellularization is an alternative way to customize an organ and transplant it without having the host versus graft rejection of an organ [28] which was first described in 2008. The 3D bioprinting is advanced manufacturing technology applied for

scaffold production for the generation of complex structures which is used to regenerate damaged tissue. The regenerated tissue via 3D bioprinting has enhanced features such as better permeability, physical and mechanical properties, and fine cellular linkage thereby providing numerous improvements over the established methods [29]. The process of decellularization and 3D bioprinting has been discussed in detail in the following section.

Decellularization: Methods of Whole-Organ Decellularization

Decellularization is a process of removal of the DNA, lipids, soluble protein, sugars, and other cellular components from tissue. It is a promising and reliable technique that can be applied to any cadaveric solid organ. The schematic process of decellularization is shown in Fig. 2. This process begins with a harvested organ which is recently derived from the brain-dead patient and is placed into a specialized vessel to generate the pale white matrix, i.e., extracellular matrix (ECM) with a complex mixture of structural and functional proteins to preserve structural entities, such as collagen, elastin, glycosaminoglycan (GAG), and fibronectin which function as the scaffold. The matrix can be obtained by employing the decellularization agents which include chemical, enzymatic, and physical methods [30–43].

Decellularization technique has proven potential for organ synthesis in tissue engineering as it keeps alive the 3D structure of organs and ECM (in between which cells are entrapped and are difficult to manufacture). Stripping away the donor's cells causes the removal of antigens that may elicit an immune response and this potentially eliminates the use of corticosteroid treatment.

To build the structural and functional organ, the process of decellularization starts with the procurement of the ECM which can be done in two ways [44, 45]. Firstly, by culturing the cell in vitro conditions to build the scaffold and secondly,

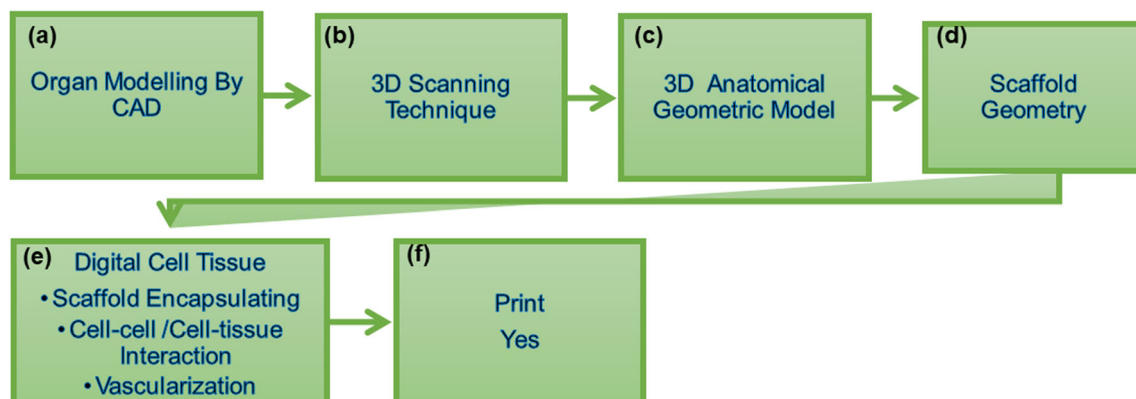
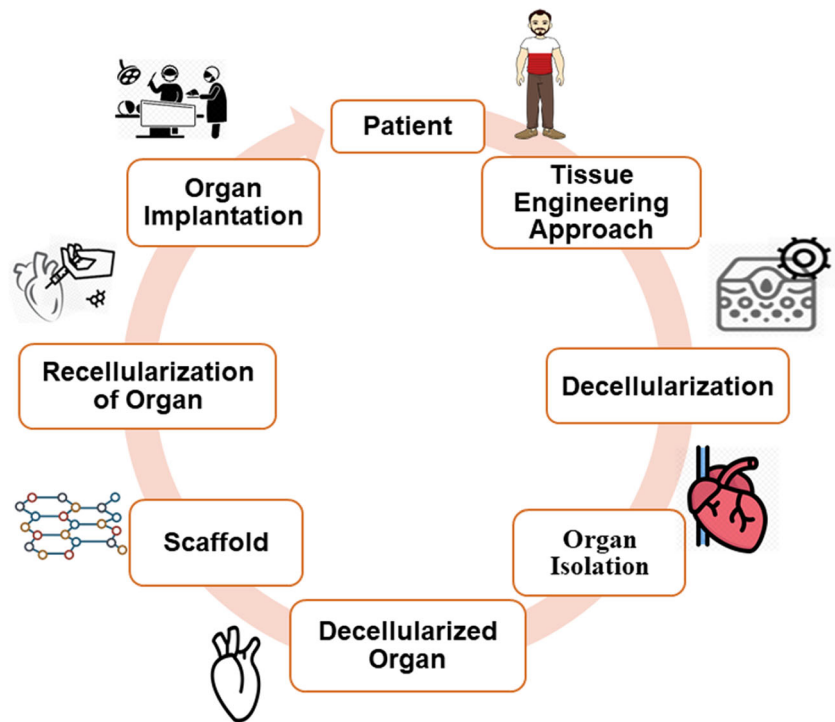


Fig. 1 Flowchart showing steps of scaffold designing: (a) computer simulation and modelling using CAD software. (b) MRI scanning of organ. (c) and (d) Anatomical and geometric designing of 3D structure according

to tomographic information. (e) Scaffold fabrication and biomaterial and cell suspension selection (f) print the model

Fig. 2 Process of decellularization: bioartificial organ development using decellularization and recellularization approach and its probable application



via surgery. While getting scaffold surgically, one should keep in mind some important aspects such as organ age, organ source, sterilization and storage, pressure and temperature, and enzymatic activities. The commonly used decellularization methods are described in Table 1. These methods include the use of detergents, acids, and bases that remove all the native cells and enzymes that can cause rejection during transplantation. Furthermore, after decellularization, i.e., stripping away the donor's cells, the process of recellularization of the decellularized ECM started to produce the structurally and functionally activated organ [45, 47].

Recellularization

The process of recellularization involves repopulating the decellularized ECM with stem cells. Certain factors are crucial for repopulation such as the number of cells, route of infusion, method of infusion, pressure, growth factors, and oxygen supply [47]. As the organ has been recellularized and is ready for the transplantation process, the assessment of recellularized organs is being done which includes steps like determining the functional characteristics, types of cells generated, and most importantly survival rate of cells. After the assessment and repopulation of cells with the recipient's very own stem

Table 1 Techniques used to decellularized tissues and organs

Decellularization methods					
S. no.	Types of methods			References	
1	Enzymes	Trypsin	Endonucleases	Exonuclease	[32–34, 38]
2	Physical	Mechanical Agitation	Freeze/Thaw	Sonication	[28, 32, 36, 38, 40]
3	Chemical	Alkaline/acid	Hypertonic/Hypotonic	EDTA EGTA	[37, 39, 42, 43]
4	Detergents	Non ionic •Triton X-100	Ionic •Sodium Dodecyl Sulfate •Triton X-200	Zwitterionic •CHAPS •Tri (n-butyl) phosphate	[28, 30, 46]

cells, the generated bioartificial organs have a little or no chance of rejection. The recellularization comprises two major steps: (1) cell seeding in which cells are incorporated into decellularized organ mimicking the *in vivo* configuration and (2) perfusion culture which involves the preparation of organ or tissue infused with cells for *in vivo* functions. Cells are repopulated with an acceptable mixture of cells inserted into the scaffold to balance the *in vivo* cellular structure while cell seeding [48–51]. This method involves either direct infusion of cells into the organ via injection or infusion of cells into the circulation with the expectation that the cells will reach the injury site. Continuous perfusion is performed after the intramural injection of cells or infusion of cells into the vasculature [46, 52]; however, thrombosis limits the cellular repopulation in the ECM scaffolds. In contrast to this, in the perfusion culture method after repopulating the cells, the organ is ready to the perfusate. The perfusate has been selected based on the culture media used in the cell culture of the constituent cell types. By employing this approach, primary goal of viable organ culture can be achieved within few weeks [53–55]. However, certain factors such as difficulty in the procurement of all ECM, immunogenicity, inhomogeneous distribution of cells, organ viability, and age after repopulating it with stem cells, organ structural complexity, and high cost limit the application of this method.

3D Bioprinting

Bioprinting is a layer-by-layer additive fabrication process that uses living cell suspension instead of thermoplastic or resin to create three-dimensional structures. In the pharmaceutical industries and tissue engineering, to construct 3D structure, the bioink or biomaterials which contain cells and growth factors is layered from bottom to top [56]. Recent advances in 3D bioprinting have the potential to meet the demands of tissues and organs for transplant by printing them. The steps involved in the 3D bioprinting process are presented in the schematic shown in Fig. 3. The first step is to model the 3D structure of tissue or organ using a computer modelling

program followed by printing with bioink producing 3D structures. Subsequently, post-processing and assessment of the physical, mechanical, and biological function of 3D-printed tissue have been carried out before transplanting the printed organs or tissue in the patients. The 3D bioprinting can be used as potential approach to meet the demand of organs for critical human organ transplantation cases [57].

The 3D printing technique was first developed in the 1980s using computer-aided software (CAD) to develop 3D structures; however, the organ and tissue printing evolved in later stage. More recently, the 3D bioprinting method has grown as multidisciplinary technology converging various areas such as bioinformatics, chemistry, mathematics, computer sciences, medicine, material sciences, biological sciences, and industrial designer which makes this widely applicable. 3D bioprinting is rapid and a manufacturing technology that is used to fabricate the complex shape with precision and this process builds this architecture layer by layer [58]. 3D printing involves complex protocol to be processed as it includes the additional planning for printing multiple cells, including the printing of vasculature and neural network for actually developing the fully functional 3D bioprinted organ [59, 60].

In this technique, bioprinter-type, bioink selection, cell-type, nozzle-type, 2D, and 3D imaging of the organ, modeling, and designing of organ/tissue and microenvironment for culture must be chosen properly for better printing of tissue [56]. The biofabrication of tissues and organs in a stratified manner is divided into three steps: (a) pre-bioprinting (modeling), (b) bioprinting, (c) post-bioprinting (maturation and assessment), which is shown in Fig. 4.

In the pre-bioprinting process, the appropriate biomaterial has to be selected first for the organ that the printer is supposed to be created. For this, the 3D imaging and biopsy of the targeted organ or tissue have been performed using technologies like 3D scanners, CT, and MRI. This 3D imaging provides tomographic and architectural information. Based on these 3D images, computer-aided software generates a 3D structure of the organ. Followed by this, the specific bioink (biomaterial) was selected based upon the type of bioprinter and the properties of the targeted tissue [61]. Selecting the

Fig. 3 Hierarchical procedure of designing, printing, and post-printing assessment

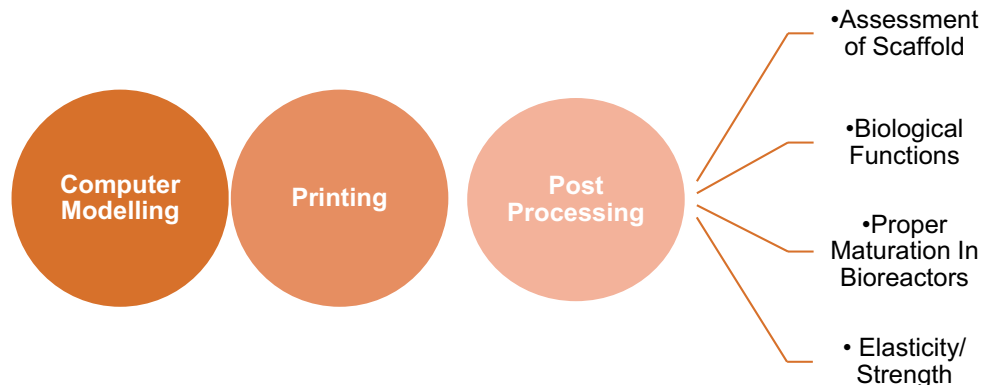
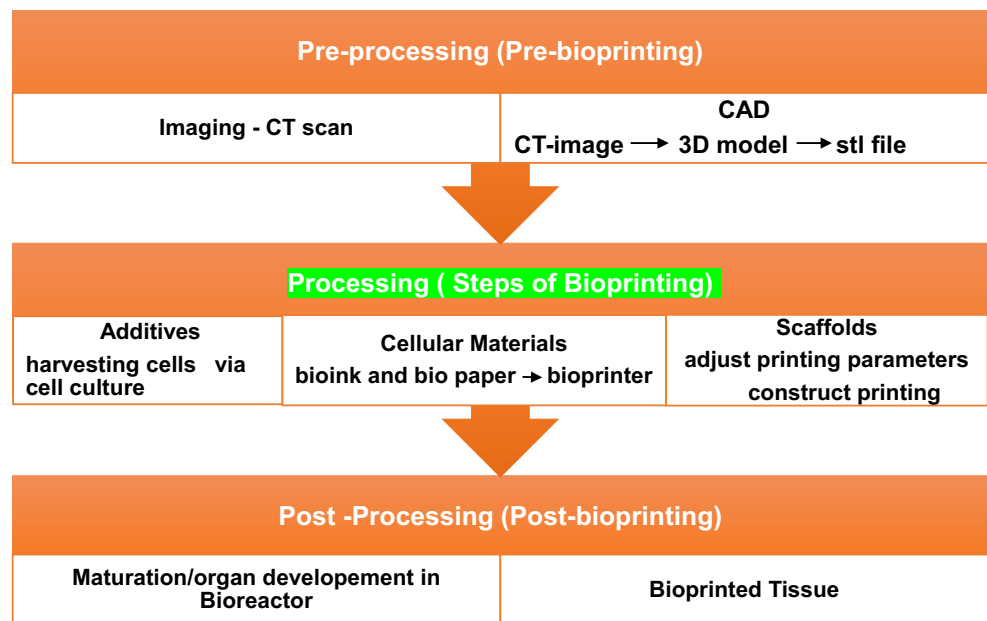


Fig. 4 Steps In 3D bioprinting: different steps and stages that lead to the production of bioprinted constructs for implantation or in vitro testing



cost-effective suitable biomaterial that could provide both physical and mechanical strength is a crucial step. Furthermore, the bioprinting process requires a 3D design of the organ, cells specific to the patient, and an appropriate bioink. Initially, the 3D design, cells, and bioink are directly loaded to the printer which is followed by layer-by-layer deposition of the cells. The printer gradually deposited layers of liquid, cells, and other required material to create the 3D structure of the organ as per command given by the software which generally recognized “STL” file format. The printer is equipped with nozzles consisting of multiple biomaterials inks, at adjustable angles [56–63]. Bioprinting can be divided into two parts: cellular bioprinting and acellular bioprinting. Cellular bioprinting is a technique that involves the fabrication of tissue with living cells. Based on printing, i.e., deposition of bioink the cellular bioprinting is further divided into 3 types: droplet-based, extrusion-based, and stereolithography. In the case of the acellular bioprinting method choice of various types of bioink for a bioreactor to obtain the cells, seeding is available. This is further divided into two types: extrusion-based acellular bioprinting (EAB) and laser-based acellular bioprinting (LAB). The types of cellular and acellular bioprinters have been discussed in the subsequent section of this review.

The post-bioprinting stage involves the maturation of printed organs which includes the development of the neural networks, extracellular matrix (support mechanically), and natural functions. After this, the bioprinted organ is further assessed through bioreactors that are required for creating higher mechanical elasticity of the tissue, assessing biological functions, and an accurate scaffold that is completely ready for in vitro usage [56, 57]. Post-processing steps are performed on the final product that includes removing the unconsumed

fragments, cooling, drilling, cutting, polishing, and sterilization. Also, the end products are being tested to make sure the product has appropriate characteristics or traits that include all its functional properties and strength [64, 65].

Type of 3D Printers

As mentioned in previous section, 3D printing is an automated method that can be explored for modelling and designing complex 3D structures (outer geometry, pore, and internal connecting vessels) by using 3D bioprinters. The scaffolds and extracellular matrix along with specific biomaterials are used as precursors for printing organ. In this technique, in vivo environment under in vitro conditions has been provided by simply mimicking the situations [66]. The schematic in Fig. 5 shows types of 3D printers. In this article, we will discuss commonly used 3D printers which include microextrusion printers, laser-based printers, and inkjet printers. The specific features such as resolution, speed, volume, stability, the viscosity of bioink, bioink type, cell density, and cost of five different types of bioprinting techniques are discussed in Table 2.

Extrusion-Based Bioprinting

This technology is rapidly expanding and has made significant growth in the last few decades. In this method, 3D printing is done by exerting the mechanical pressure, and the bioink is extruded out through the heated nozzle onto the printing bed [66, 67]. These types of printers are used for printing various structures including cells, tissues, tissue constructs, modules, and microfluidic devices. Despite of several advantages and

Table 2 Specific features such as resolution, speed, volume, stability, the viscosity of bioink, bioink type, cell density, and cost of five different types of bioprinting techniques

Characteristics	Inkjet	Extrusion	Laser-assisted	Stereolithography	Fused deposition modelling	Reference
Resolution	50–300 μm	Wide range	Microscale resolution	High	Low	[21, 23]
Speed	Fast (1–10,000 droplets s^{-1})	Slow (5mm s^{-1})	Medium (100 droplets s^{-1})	Slow	Fast	[32]
Volume (capacity of bioink storage)	ml range	ml range	>500ml	Up to 300 x 335 x 200 mm	Up to ~200 x 200 x 300 mm	[23, 31]
Stability	Poor	Good	Fair	Good	Fair	[35, 36]
Viscosity of bioink	3.5–12 mPa/s	up to above 6×10^7 mPa/s	1–300 mPa/s	No limitation	5 mPa/s	[32, 35, 36]
Bioink type	Cell suspension	Viscous materials	Cell suspension, Viscous materials	Photosensitive Resins	Thermoplastics such as PLA, PLLA, Nylon, PCL, PLGA	[37, 38]
Cell density	Low ($<10^6$ cells ml^{-1})	High (cell spheroids)	Medium	Medium / no limitation	low	[31]
Cost	Low	Medium	High	High	Low	[28, 29]
Specific characteristic	Materials used are low cost; contactless sterile printing	Commercially available	Compatible	Limited material	Limited material	[25, 26, 31, 32]

great versatility in printing the various 3D structures, many questions and restrictions are being raised such as organ fabrication and low resolution of printed features [68, 69].

Microextrusion Printing

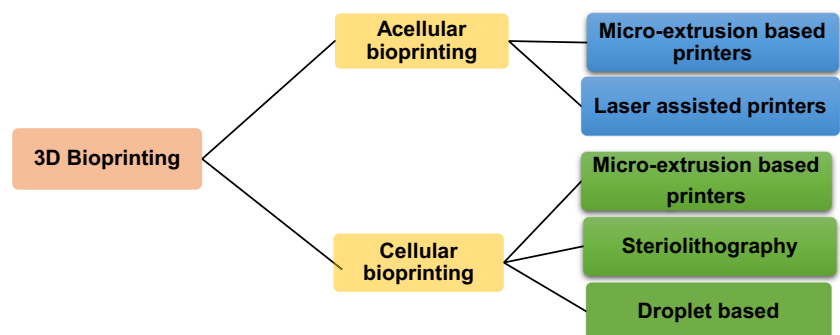
This printing technique is based upon the principle of extrusion, i.e., it uses force to selectively divide the bio-material and cells via nozzles and needles with the application of heat [67]. Microextrusion bioprinters usually consist of a temperature-controlled biomaterial dispensing system which generates a constant string of bioink rather than many droplets of bioink by applying pressure (pneumatically or mechanically). A microextrusion printer consists of numerous nozzles for each material to be used for designing the architecture, but certain factors should keep in mind such as enough space between the nozzles, speeds, diameter of the nozzle, and the mechanical force to inject material via the piston. Furthermore, the

viscosity of bioinks used in the printer must be appropriate, as it maintains the architecture of the scaffold and prevents it from collapsing while maintaining the high-resolution printing Fig. 6.

Inkjet Printers

This technique is based on the working of general 2D printers. This printing technique involves the heating principle. In this, a piezoelectric actuator vaporizes the cell suspended liquid into bubbles which can escape through a nozzle in droplet form from chosen position [70]. It has high printing speed but due to the use of a piezoelectric actuator, the cells come in direct contact with the heat, which results in cell membrane damage and cell lysis. However, the inkjet bioprinting technology can hasten the study of tissue biofabrication [71]. There is remarkable success in the invention of bioinks and biopapers that are appropriate for inkjet bioprinters. This technology allows the printing of complicated organs and tissue

Fig. 5 Hierarchy chart of bioprinting techniques: (A) First division shows the types of bioprinting techniques. (B) Second division depicts the type of printers



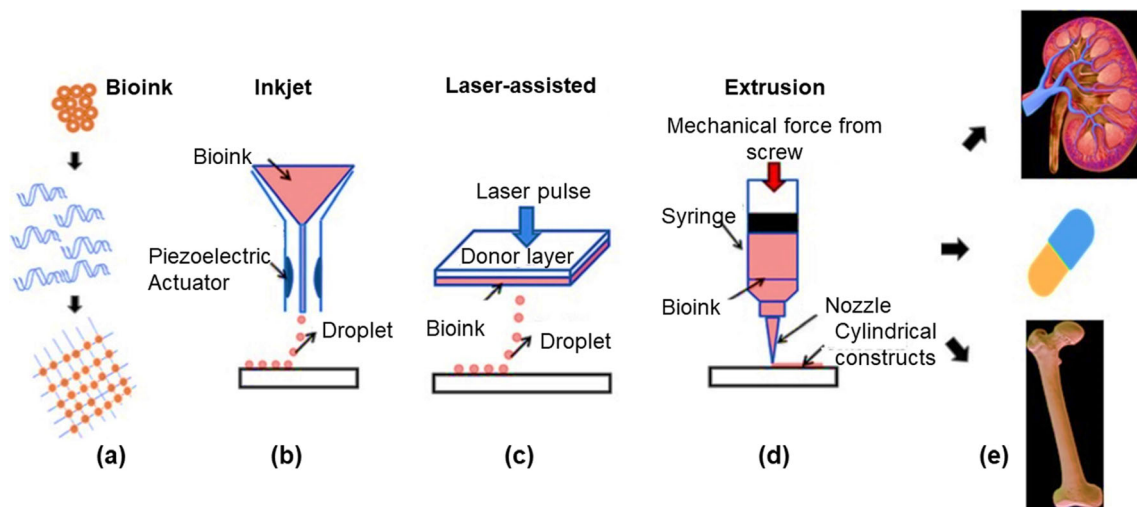


Fig. 6 Type of bioprinting approaches and their working methods. (a) Workflow of designing the bioink for printers involves the living cell suspension and biomaterials. (b) Inkjet-based printing extrudes out the fractions of molecules of bioink. (c) Laser-based bioprinting involves the use of laser focused on absorbing layer, high gas pressure generated by

affected area induces the bioink onto the printing bed; (d) Extrusion-based bioprinting uses mechanical pressure to frequently extrude out bioink (cell-polymer solution). finally, the scaffold is made layer by layer. (e) The medicinal approach in tissue engineering, drug testing, and designing for in vitro study model for disease diagnosis

with accuracy by using the cells as bioinks, although it has limitations such as large organs cannot be printed properly and the droplet size cannot be controlled. However, it is a much-preferred technique over microextrusion technique as it provides enhanced resolution of droplets and exhibits great cell viability [72].

Laser-Based Printers

This printing technique uses lasers for designing the architecture of the tissue. The use of lasers provides the droplets with high resolution. Initially, the laser is focused and bioink is cast on the substrate or printing bed. The laser beam gets activated immediately after striking the absorbing layer, the laser impacted area evaporates and the high gas pressure induces the bioink onto the printing bed, and finally, the scaffold is made layer by layer. The laser printers are advantageous when compared to microextrusion and inkjet printers as it uses less force and minimum heating while printing the tissue [73].

Stereolithography Bioprinting

This is the oldest approach for bioprinting 3D structures with high resolution and precision [74]. This bioprinting technique produces complex 3D structures by frequently layering on 2D-lined layers afterwards the photo-polymerization of 2D patterned layers is considered to be the most crucial step in this bioprinting [75]. The models and fabricates made with this technique are well-built and can be used to make master models for various metal casting processes [75–77]. This

technique is costly and the photopolymers used in this technique need to be handled with care as they are more viscous [78, 79].

Droplet-Based Bioprinting

Droplet-based bioprinting technology is all-purpose technology as it offers higher resolution and allows the better regulation of geometry and size of bioprinted 3D structures. This technology possesses greater resourcefulness in various fields of studies that attracted many researchers to use this technology in the field of bioprinting [80, 81]. Its affordable commercial availability and versatility [82] make it the technique of choice. However, a small diameter of the orifice of this printer causes the clogging of the orifice during the bioprinting and the bioink keeps accumulating within the orifice and results in obstruction of flow which limits its application. Due to this reason, instead of bioinks, hydrogels and other biomaterials are used for constructing the structures [83]. Besides this, droplet-based bioprinter fails to assemble mechanically strong and structurally unified constructs due to the restricted selection of biomaterials [84]. Moreover, despite the high resolution of droplet-based bioprinter, it is a much time-consuming technique that sometimes results in swelling of the tissue structure [85, 86]. Among all the 3D bioprinters described above, laser-assisted or laser-based bioprinters are more successful and widely used to fabricate organs and tissues [73]. Unlike extrusion, microextrusion, and inkjet printers, these 3D bioprinters do not use a nozzle to dispense ink; instead of this, they use a direct UV light on the printing bed of bioink which gets hard when exposed to light.

Bioinks: the Building Material

Additive manufacturing technologies–based 3D bioprinting techniques have made significant progress in recent years for the development of complex geometry. In this process, selection of appropriate bioink, cells, and biomaterials for the effective fabrication of targeted tissue plays crucial role. The successfulness of the fabrication depends upon properties such as solidifying kinetics of the biomaterials and the native, chemically, or environmentally induced material properties [87]. A suitable bioink material should provide the growth factors, adhesion factors, signaling proteins, and mechanical and structural properties of ECM. These are the progressive events that promote a suitable environment for cell survival, viability, and differentiation [88]. The conventional techniques used were not able to build structures with the expected interest and desires. While modeling organs and tissues, bioink, biomaterial, and cells should be selected appropriately that resemble the tissue intended to be reconstructed to develop inevitably correct biological structure. The major types of bioink materials used for printing are cell-scaffold-based bioprinting and scaffold-free bioprinting. In the cell-based bioprinting approach, the bioink consists of biomaterials incorporated with the cell suspension to build the 3D construct of tissues and organs. Furthermore, the scaffold gets degraded and cells proliferate and occupy the space to form pre-designed tissue structures whereas in scaffold-free bioprinting, the bioink only consists of cell suspension. Here, the cells are programmed to behave like embryonic stem cells, which further get arranged in a specific manner to perform some specialized functions [89–93].

Biomaterial Selection

The biomaterial selected for 3D printing of tissue construct should be affordable, commercially available, and convenient for clinical purposes. Furthermore, it should be biocompatible with the biological system; therefore, it cannot elicit the immune response and provide an environment for cell survival, motility, and differentiation. Besides this, biomaterial must have suitable physicochemical properties which should meet the demands of the printer [94–96]. The hydrogel, ceramic, polymers, and composites are some excellent examples of currently used biomaterials for 3D printing. Hydrogels are the most widely used biomaterial ink for the bioprinting of the organ as it provides temporary support to cells while they produce their own ECM. These are water-swollen, cross-linked polymeric structures produced by the simple reaction of one or two monomers. The hydrogen bonds and Vander Waal interaction are generally involving in cross-linking between the chains. Hydrogel is suitable for all types of printer inkjet, laser-based, microextrusion. Hydrogels can be formed using three types of polymers: natural such as cellulose,

fibrinogen, gelatine, collagen, and agar; synthetics such as polyethylene glycol, and polyacrylamide; or synthetic-natural mixture. Hydrogels are commonly used for bone regeneration or bone defects coating with hydroxyapatite, carbon nanotubes [94, 95]. Ceramics are also used frequently as biomaterials for printing because of their unique properties such as hardness, brittleness, and chemical inertness. Most importantly, ceramics are biocompatible and have a similar composition as bones. Ceramics are generally used in inkjet- and particle-based bioprinters which convert the powder into suspension form. In powder bed-based printers, the ceramic powder of appropriate size (diameter 10–150 μm) and proper particle flow within the bed is used. However, for inkjet printers, apart from particle size, viscosity, and solid content of ceramics, biomaterials should be appropriate. Ceramics are used for the replacement of hips, knees, teeth, tendons, and ligaments. Also, it is commonly used by doctors for facial and maxillofacial reconstruction [97, 98]. Hydroxyapatite-based ceramics are highly bioactive and biocompatible materials and have a similar composition as bones. Certain polymers are also used in bioprinters due to their low cost and other suitable properties such as biodegradability, flexibility, good mechanics, and biocompatibility. Polymers are easily manufactured by printers and can be transformed into any desired shape. Generally, polymers are used by dentists and pharmacologists for drug delivery and targeting sites of inflammation or tumors [91, 92]. Some composites are also used as bioinks due to their excellent properties such as bioactive, stiffness, and printer compatibility. The most commonly used composites for the printers are biomolecules, carbon nanotubes, and some metal and alloys [97].

Selection of Cell Lines

Multiple cell types which are structurally and functionally specific generally constitute an organ or tissue and all these cells exhibit regenerative capacity. Therefore, while selecting the cells for the organ printing, certain factors should be kept in mind such as the cells must have appropriate proliferation and differentiation capacity in printed scaffolds along with the ability to involve in the signaling cascade. Furthermore, the cell survival and viability during and after the printing procedure are crucial factors; hence, enough cells should be cultured and taken for the printing process. Cellular functions should be checked and maintained in *in vitro* as well as *in vivo* environments [98].

For 3D printing of desired tissue construct and organ, bioink, biomaterials, and cells should be chosen wisely keeping all the above-mentioned crucial factors in mind. Several organs have been successfully printed and transplanted in patients listed in Table 3. Table 3 discusses the organs printed by a different type of 3D printing technique and the types of bioink used for the printing

Table 3 Different 3D printing technique for bioprinting of various organ and tissues

Target organ/ tissue	Printing technique	Bioink	References
Skin	Droplet/inkjet	i Cell suspended media for printing cells ii Collagen solution for printing the supportive layer iii Hydrogel	[99–102]
	Laser assisted	Matriderm	
Liver	Inkjet	i Cell suspended media for printing cells ii Fibronectin gelatine solution for printing adhesive film between monolayer	[103–105]
	Stereolithography	Gelatine methacrylate for printing hiPSC	[106]
Bone	Extrusion	Hepatocyte aggregates	[107]
	Extrusion-based rapid EBM	Natural and synthetic polymers solution	[108]
	Prototyping (RP)		
	Fused deposition modelling (FDM)	Synthetic polymers, such as acrylonitrile butadiene styrene (ABS), polylactic acid (PLA), Polyvinyl alcohol (PVA)	[109]
	SLA/SLM	PPF/DEF-HA, PDLA/HA, β -tricalcium phosphate (β -TCP)	[108–112]
	Binderjetting, EBM	HA, Zirconia, HA/MG63 osteoblast-like cell, nano HA, human osteoprogenitor cell, Human umbilical vein endothelial cell	[112]
Cartilage	Pneumatic	Natural polymer solutions, such as alginate and proteins, cells, and growth factors can be incorporated	[113, 114]
	Extrusion based		
Ear craniofacial	Extrusion-Based -Rp	Natural or synthetic polymers solution	[115]
	Direct write RP	A concentrated colloidal gel (typically 50% of HA in an aqueous medium)	[116]
	Stereolithography	Polypropylene	[116, 117]
	Inkjet	PEEK, PLA, PLGA.	[117]
	Extrusion (EBM)	Fibrinogen, Gelatin, Alginate	[117, 118]
	FDM	Titanium, PCL	[118]
Trochlea (rabbit)	Indirect microstereolithography	Poly- (L-Lactide-co- ϵ -caprolactone)/gelatine, heparin, transforming growth factor beta 1, chondrocytes	[119]
Bone defects	FDM (fused deposition modelling)	Hydroxyapatite incorporated polycaprolactone	[111, 120]
	Binderjetting, EBM		
Endothelial progenitor cells	Thermal inkjet-based AM	Collagen solutions	[121]
Breast cancer	Extrusion	i Breast cancer cell aggregates	[122]
		ii Stromal cell aggregates	
Cervical cancer	Extrusion	Gelatine -alginate-fibrinogen solution for printing cells	[123]

process. Printed tissues can be of two types: soft tissue such as muscles, tendons, ligaments, nerves, blood vessels, synovial membrane, skin, and fascia, and hard tissue such as bones, tooth, cartilage, and enamel. The hard organs for instance artificial limb, bladder, brain, testis, eye, lungs, ovaries, pancreas, trachea, thymus, heart, kidney, liver, and ear can also be printed successfully using this technology.

Applications of 3D Printing Techniques

Organs generated by 3D printing technologies enable patient's recovery faster and more efficiently. Recent advances in the field of 3D printing have gained considerable attention as it can suffice the need for organs that cannot only improve the quality of life of patients but also save lives in many cases. Besides organ printing, the 3D printing technology offers

extended medical applications such as synthesis of prosthetics, drug screening, testing, and manufacturing of good-quality cosmetics. Notably, this technology can help generate personalized medicine for the cure of specific disorders in patients. Personalized medicine reduces the possibility of adverse effects due to these dosages outside the therapeutic window or subtherapeutic benefits.

Clinical Application for Organ/Tissue Printing

The bioprinting process utilizes the patient's cell for constructing the fully functional three-dimensional tissue construct by assembling cells layer-by-layer by using biocompatible scaffolds which when incorporated with living cells regenerate functional tissue.

The computer-aided design software has been used for effective 3D bioprinting to build the 3D constructs. All the processes in this technique such as position and speed of printing nozzles, and deposition of cells and materials are strictly controlled by the software. Some of the examples of organs generated by the 3D printing technique are listed in Table 3. However, this technology can be used to create all types of organs or tissue and some of the successful 3D printed organs are as follows

Skin Bioprinting The imminent solution to burn wound reconstruction is the utilization of artificial skin which can cure the skin of third-degree burns [124, 125]. The third-degree burns or secondary burn injuries required surgical ablation of damaged skin and replacing it with the traditional and accepted skin which can be successfully achieved by stratified 3D bioprinting technique. In this process, desired scaffolds are incorporated with living cells over the burn parts, which heals the burn via the formation of healthy skin tissue. In this regard, clinically convenient broad-spectrum skin substitutes are available, e.g., Matriderm®, Integra®, Dermagraft®, and OrCel® [126, 127]. To construct the bioprinted skin, multiple cell types and biomaterials are preferred. Initially, the scaffold of dermal and epidermal skin is incorporated with keratinocytes and fibroblast; after that, they are cultured at a liquid interface that gives rise to the skin appearance. The melanocytes are generally printed between the layers to have a complete biometric skin [99–103, 128]. In a recent study, bioink is prepared by mixing silk protein with gelatine and then blended with specific skin-forming cells [102]. However, certain skin appendages like sweat glands, melanocytes, endothelial cells, hair follicles, and sebaceous glands in the skin are still troublesome to create. Despite this fact, artificial skin developed by the 3D printing technology has proven effective in wound healing and is also used in various pharmaceutical companies and chemical testing researches [103].

Hepatic Tissue Bioprinting Liver injury and hepatic diseases such as fatty liver and hepatitis have increased the demand of hepatic tissue bioprinting [104]. Nguyen et al. have bioprinted 3D primary liver tissues that facilitate the evaluation and testing of organ level response of drug [105]. In another study, Chang et al. biofabricated micro-3D liver organ, which can be explored as in vitro drug testing and to study metabolism [129]. A simple one step fabrication approach of an organ-on-a-chip using 3D printing has been described by Lee et al. [106]. A liver-on-a-chip platform with bioprinted hepatic spheroids has been demonstrated in another study [107].

Cartilage Bioprinting Cartilage tissue is a type of elastic connective tissue; if it gets damaged, it is incompetent to reconstruct on its own and therefore it is surgically restored by a substitute. However, artificial substitutes failed to heal naturally which causes cartilage lesions [113, 114, 130–132]. In 3D printing, cartilaginous tissue scaffolds are incorporated with bioink such as chondrocytes, mesenchymal stem cells, and bone marrow cells which led to the generation of desired tissue [113, 132]. Certain limitations like weak mechanical strength and firmness of the implanted cartilage tissue raised concern. Nevertheless, 3D bioprinting shows great applications in cartilage tissue repair as it promises to mimic the exact in vivo environment of cartilage tissue. A group of researchers in Sweden successfully introduced 3D printed human cartilage into mice [132]. They printed a hydrogel of nanocellulose mixed with human-derived cartilage cells, then surgically implanted the structures into mice. Once implanted, new blood vessels formed within the printed cartilage.

Kidney The kidney is one of the most difficult organs to be constructed by 3D printed technology because of its complex structure [133, 134]. The bioinks consisting of kidney cells and surrounding materials for printing kidneys have been used in this context. The bioink has the thickness of a toothpaste that can be expelled at room temperature and enables the building of complex structures. This research group has been able to rejuvenate the nephron, the operational unit of the kidney which can conduct filtration and reabsorption of useful components. Besides this, several alternative organs also named grafts, prostheses, implants, analogs, and precursors, and these are clinically part of regenerative medicine and are successfully generated using this technology.

There are several case studies in which 3D printed organs were transplanted successfully. A patient with spina bifida (a condition where a part of the spinal cord has grown outside the spinal column) had bladder outgrowth which results in urine leakage that affects the kidney. In this case, the patient's healthy cells were used to make up the urinary tract and bladder [134]. In another case, 3D printed medical model company along with surgeons at Belfast City Hospital in Northern

Ireland created a 3D printed replica of the kidney which works fine [135].

The Heart The heart is the easiest organ to be printed as it does not have a complex structure and its primary function is to pump blood [136, 137]. For heart tissue printing, initially, the donor's heart is scanned with CT and MRI machines which find out the structure and size of the heart. Afterward, the patients' blood cells are collected and converted into stem cells under the influence of growth factors which regenerates the heart cells using bioink mixed with hydrogel in 3D printers [136–139]. Here, the bioink is being stratified onto a scaffold that provides mechanical support and the exact shape of the patient heart where cells grow and proliferate. After a few days, cells start to join and begin to beat like an original heart. The scaffold is now removed and the heart is ready to implant. The person's stem cells are used to reconstruct the organ which reduces the chances of host versus graft disease and there would be no need for immunosuppressant drugs. A biotech start-up company BioLife4D constructed a miniature heart for testing in small animals [138]. A group of researchers from Carnegie Mellon University developed a new method for 3D bioprinted tissue scaffolds out of collagen and this new technique allows to 3D print a full-sized, adult human heart [140]. In another report, when the 3D bioprinted heart incorporated with human cells, the heart does not beat as it was too small; it was only about the size of a rabbit's heart, too small for a human [141].

Bones The new method “Nanokicking” was developed to grow 3D constructs of mineralized bones by researchers of the University of Glasgow. They used stem cells from the human donor and turning them into the 3D bone graft and this bone graft was implanted into the dog's leg. It is expected that this 3D bone graft work would be ready to transplant in humans by the end of 2020 [108, 110].

In a similar report, based on a CT scan of the patient, engineers design the 3D printed bone implants together with the surgeon which can fit perfectly in place. The process showed promising results but it is still in the testing phase [109]. The pre-clinical study showed that, within 24 weeks, the implant fused with the patient's bone started rejuvenating and marrow was beginning to form in the transplant [112]. Moreover, 3D printing of bone tissue using different compatible biomaterials has been carried out successfully [116–121].

Drug Screening and Testing

3D printing enables the quick production of customized models and manufacturing of complex structures with different geometries. The technology offers drug production and customization that is personalized-dose medicines, sophisticated and complex dosage forms, on-demand manufacturing,

and the development of drug delivery systems with non-traditional geometries for pharmaceutical drug testing applications (e.g., drug-eluting implants) [122, 123, 142–146]. The first time 3D printed tablets gained US FDA approval was in 2015; since then, 3D printing of drugs is expected to increase in the pharmaceutical industry and pharmacy practice [147]. The *in vivo* assessment through bioprinting is quick and easy. Furthermore, the customization of the drug can be regulated as the matrix properties, porosity, and shape could easily be manipulated by using different material choices used for printing the scaffold that affects cell adhesion. Therefore, bioprinting demonstrated huge potential in drug discovery and preclinical testing due to its high speed, precision, and consistent reproducibility [148].

Recently, researchers have successfully used mini tissue, organ-on-chip, and tissue constructs for *in vitro* drug screening [149–151]. 3D mini-tissue models can be constructed through common 3D bioprinting techniques based on various cell sources and biomaterials [152–154]. 3D mini-tissue models facilitate high-throughput testing in case of cancer disease which can provide predictive or prognostic information. However, the drug screening in 2D cell culture models are easy to manipulate but it lacks the *in vivo* microenvironment and therefore one cannot predict its full efficacy. In this context, the 3D printed tissue models and organ-on-a-chip mimic the complex microenvironment of human tissues and therefore drug testing efficacy can be predicted more accurately [155, 156]. Jalili-Firoozinezhad et al. investigated the efficacy of radiation countermeasure drugs by using a gut-on-a-chip model. This model consists of epithelial and endothelial cells and cyclic suction was applied to mimic the stress felt during physiological peristalsis movement by cells. Under controlled conditions, epithelial cells undergo villus differentiation, similar to that inside the human body [156]. However, a more personalized approach is needed for drug screening and testing with strict standardization and quantification requirements [157–160] which can be resolved by introducing ECM mimicking materials in tissue/organ construct. The organ-on-a-chip models produced using a tissue engineering approach have the potential to be perfect models for drug testing. It involves incorporating the cells into scaffolds and allowed to grow which created a functional construct that can be used to replace/repair damaged tissues or organs [158]. These models have the potential to replace animal models that are used for drug response and toxicology screening. By reducing the number of animals used in preclinical studies, the cost of drug development can be reduced, and also the ethical issues regarding laboratory animal usage for drug testing and screening can be compensated.

Recently, several efforts have been made to construct the tumor microenvironment of various cancers including cervical cancer and triple-negative breast cancer with fibroblasts using 3D bioprinting technologies [158–160]. The tumor

microenvironment thus created was used to study the effect of drugs on the stromal cells in cancer [160]. Langer et al. bioprinted a heterotypic tumor microenvironment that consisted of patient-derived cancer cells, fibroblasts, and endothelial cells, which closely mimic *in vivo* tumor microenvironment [161].

COVID-19 Testing and Shortage Supply

COVID-19 was first detected in China on December 19 causing an expanding and accelerating health crisis around the world. This pandemic has placed massive stress and a huge gap in demand and supply chains in terms of diagnostic kits, reagents, drugs, face masks, and PPE kits [161–164]. The 3D bioprinting-based additive manufacturing appears to be an attractive solution and holds promise to combat the shortage of these supplies. Using 3D printing, the fabrication of complex structures can be done and the printing can be adjustable for the production of the respiratory mask, face shields, ventilator valves, testing kits, and other equipment that are desirable during this pandemic [162]. However, standard safety and quality measures of medical devices should be regulated and taken care of in 3D printing labs to ensure the goodness of fit of the bioprinted materials for human use [159] which can be improved through education, monitoring, and quality testing of procedures.

In the current pandemic, 3D printers have been used for rapid design and development of the equipment and testing kits which include a nasopharyngeal swab, personal protective equipment, and face shields. Moreover, an alternative approach for drug production and validation was done by multi-compartment and multi-layer 3D printing which was used for developing variable or fixed-dose combinations of two or more anti-viral therapeutics [164, 165]. The cost-effective nasal swabs were designed and printed using polyethylene terephthalate glycol (PETG) filament by major type of filament-based printer at the Department of Pathology and Microbiology, University of Nebraska Medical Centre, Omaha, NE, USA. These swabs are durable, chemically inert, and well suited for structural applications [165]. Similarly, an inkjet-powder bed printer was used for drug loading as it dissolves drugs quickly and easily therefore can lower the drug administration burden for critically ill patients. This enhancement of drug solubility using FDM filaments which are created with ASD formulations is done by locking the active drug compounds into a formulated filament. This reduces the potential misuse of drug treatment during the panic of an ongoing crisis [164, 166]. Furthermore, 3D printing has already been used to produce respirator valves for the ventilators in Italy [167]. The 3D printable medical resources substantially reduce the morbidity and mortality in patients during the COVID-19 challenge. However, the cost of 3D printers and synthetic bioinks, and the low viability of cells with a lack of

vascular networks are certain drawbacks of 3D printing techniques that need to be resolved. Another limitation of this technique is the use of a synthetic scaffold that does not mimic the cells' environment; however, this can be overcome by using the naturally derived extracellular matrix (ECM) through the process of decellularization.

Future Perspective

The 3D bioprinting technique is a boon to patients having end-stage organ failure. These techniques can also be used for regenerating amputee organs and help the person to live a normal lifestyle who lost the organ or limbs accidentally. Apart from this, it can be successfully implemented in the field of personalized regenerative medicine and tissue engineering. The advances in the designing of bioengineered scaffold/extracellular matrix such as biocompatible biomaterial with minimum toxicity in the patients, angiogenesis promoting growth factors, biosensors in bioengineered scaffold which provide a timely update before perfusion of scaffold or extracellular matrix the cells and the ability of genetic manipulation of cells to prevent the disease have made these techniques most attractive and feasible to use. However, 3D tissue construct with precisely defined structures and geometries can be achieved using 3D printing techniques but these structures are not much stimulus-responsive which limits its application. Recently, 4D bioprinting and xenotransplantation have gained considerable attention in this regard. 4D bioprinting was first introduced in 2013 and since then it is rapidly growing. 4D printing technology offers a great ability to fabricate complex and stimuli-responsive 4D structures. In this technology, the 3D structure of an organ self-transforms immediately after printing when it is exposed to ultraviolet light, current, heat, or other energy sources or the predetermined stimulus, including osmotic pressure and exposure [168]. Skylar Tibbets in 2014 at the Massachusetts Institute of Technology (MIT) demonstrated multi-material printing that can change from one shape to another using 4D printing technology [169]. The homogenized tissue developed by 3D printing technology can be transformed into a fourth dimension using 4D bioprinting [170]. The five important factors taken in to account in the process of 4D printing are (1) mathematical modeling of the material transformation, (2) type of stimulus, (3) type of responsive material, (4) type of additive manufacturing process, and (5) interaction mechanism between stimulus and the material [171].

For successful 4D printing of tissue, numerical and theoretical models are mainly used. These models predict the time required for the structure to reflect the response by making links between material and stimulus properties, sequence of stimuli structure, and the desired final shape [172]. 4D printing technology involves multi-material printing that is stimuli-responsive materials, the shape of the material changes over time due to the reaction to temperature, pH, humidity, electricity, magnetic field, light, acoustics, or a combination of

these stimuli [173, 174]. 4D bioprinting has diverse application in various fields which includes biomedical, tissue engineering, biosensors, robotics, and bioactuators. The fabrication of smart and multifunctional materials of biological relevance using 4D printing technology has been reported in recent studies [175, 176]. The *in vitro* synthesis of biomimetic blood vessels using self-folding polymers which help blood vessels to incorporate into various cell types are successfully constructed using 4D printing [177]. Besides this, 4D bioprinting enables the allocation of distinct elements that can self-fold or self-unfold to encapsulate and release drugs or cells in a programmable manner to the target organ [178]. Another interesting application of 4D bioprinting technology is to cure wounds. For this, human adipose-derived stem cells are infused into shape memory polymer scaffolds, in which cells upon injury in scaffolds produced mineral deposition, protein production, and gene expression thereby healing the damaged tissue. The feasibility of printed shape memory scaffolds for bone repair has been proven experimentally [179].

Another advance in the field of tissue engineering is xenotransplantation [180–184]. A recent study done by Vunjak-Novakovic and her colleagues is the classic example of xenotransplantation. They had revived the donor lung (human) using pig (circulatory system) as a recipient. They obtained lungs that had been rejected for transplantation from six human donors, both single lungs and pairs. The team united each lung to the circulatory system of an anesthetized pig with tubes nourishing the blood vessels of the human lung from those in the neck of the pig. The circulatory system was inserted with immunosuppressant drugs which infiltrate both the pig and the human lung. After 24 h, the team observes the lungs are modified. This study also stated that the process will not protect potentially harmful pathogens diffused to people. Furthermore, it was observed that the lungs are wholly free of pig cells. However, the lungs were noticed to have lymphocytes from the pigs. The outcome of this study provides a hope that lung recipient uses their blood supply to revive donated lungs that they will receive which could increase the number of lungs available for donation to patients by three times [184].

Currently, clinical xenotransplantation trials are in progress to detect the inherently engineered finest pig either as a single organ donor or multiple organ donor. However, it is uncertain whether a specific genetically engineered pig would be ideal as a source of numerous different complex organs or it would be necessary to have organ-specific genetically engineered pigs since organ-specific heterogeneity and immunogenicity play important role in the acceptance of transplanted organ [182]. In this regard, 3D bioprinting in combination with bioinformatics and computational biology can be proven less expensive and a quick way to get desired tissue or organ. However, there are several persisting pathobiological and physiological obstacles that must be conquered before worldwide acceptance of this technology [183].

Commercially Available 3D Bioprinters

Technological advancements are projected rapidly owing to the growing use of 3D bioprinting not only in the field of mass production of organs and tissues to overcome the organ shortage but this is also exploited in cosmetic surgeries and praises a lot in pharmaceutical industries. Currently, the 3D bioprinting global market is in its developing phase and has a huge opportunity to spread in developing countries. So far, only a handful of companies enlisted in Table 4 are in the market. 3D bioprinting technologies are still in their early stages in the field of biomedical research and development.

Conclusion

This review focused on additive manufacturing techniques specifically 3D bioprinting and decellularization followed by recellularization which hold promise to combat shortage of organs and can efficiently deal with several problems in the medical field. These techniques can be exploited for generation of customized organs for the patients facing end-stage organ failure. These approaches can be successfully implemented for personalized and regenerative medicine and thus can be boon for society and human welfare. The 3D bioprinting working principle is similar to 2D printers in which instead of using paper and colored ink, specific material such as hydrogels, biomaterials, stem cells, or patient-derived cells have been used along with file in the STL format to create a tissue or organ. The most common bioprinters in the field of tissue engineering and 3D printing technology are laser-assisted printer, microextrusion, and inkjet printers which basically use different biomaterial and bioink. These biomaterials are compatible with printers and specialized to properly fabricate the tissue/organ construct. In contrast to this, decellularization is used to customize or personalize the donor organ for the patient via removing all the cellular components and the genetic material to get the naturally derived extracellular matrix called scaffold. The more personalized and natural extracellular matrix can be obtained using 3D bioprinting and decellularization approaches that can be adapted to body without eliciting much immune response in contrast to synthetic matrix where chances of rejection are higher. However, stability and natural shape of organ along with cost of techniques are few factors which limit the applicability of these techniques. Besides, the ethical approval of governing bodies of countries in terms of human organ culture and usages would be another major challenge in this regard. Nevertheless, recent advances in 4D bioprinting have ability to enhance the structural stability and shape of the tissue/organ construct. Moreover, xenotransplantation has potential to revive the donor organ without transmitting the xenogeneic disease in human recipient. Taken together, these techniques if exploited precisely and appropriately can deal with the problem of shortage of organs in the field of medical sciences.

Table 4 Commercially available bioprinters

S. no.	Company	Name of 3D printer	Specialization	Specification	Remark	Reference (web link)
1	ORGANOVO	NovoGen MMX Bioprinter	Uses human cells and shapes them into real tissue. The printer ejects a liquid filled with thousands of human cells, guided by lasers. The process repeats, creating hundreds of layers of cells, held together with a gel. The cells begin to grow, meshing themselves together, as they would in the body, and into actual tissue	Technology: syringe-based extrusion Materials: cellular hydrogels	world's first commercial 3D bioprinter to print human tissues.	https://www.invetechgroup.com/case-studies/organovo-novogen-mmx-bioprinter/
2	CELLINK	BIO X	Bioprinted tissue can be used in drug discovery where researchers can test new potential treatments and evaluate efficacy in very early stages.	<ul style="list-style-type: none"> •Layer thickness:50 - 300 Microns •Optical system: Integrated Germicidal UV C - 275 nm, Integrated UV Curing - 365, 405 nm •XYZ accuracy:2 micron •Closed print chamber: Yes, fully enclosed •Feeder system: Air Pressure Input •Extruder: Three •Print bed details: Heated bed •Weight :17kg TeVido Biodivices	The built-in features along with the new BIO X software managed through the large touchscreen display minimize the learning curve and ensure you will receive the results you want	https://www.cellink.com/bioprinting/bio-x/
3	TeVido Biodivices	TeVido's Tru Pigment	Reconstructive and cosmetic surgery	TeVido Biodivices	uses high quality, cutting-edge processing techniques to prepare transplant material – made from a patient's own living cells	https://tevidobiodevices.com/
4	Aspect biosystems	RX1 Bioprinter	Bioprinting to study and treat brain diseases.	Materials: hydrogels motion and pressure control enable microscale resolution at high speed	The Aspect RX1 3D Bioprinter enables the production of 3D heterogeneous and functional human	https://www.tpsgc-pvpsc.gc.ca/app-acq/picc-bcip/prequalified-prequalified/aspect-eng.html

Table 4 (continued)

S. no.	Company	Name of 3D printer	Specialization	Specification	Remark	Reference (web link)
5	Cyfuse Biomedical	1. Regenova 2. S-PIKE	1. Bone and Cartilage Regeneration 2. Vascular Regeneration 3. Neural Regeneration 4. Liver Structure	Weight: Total 450kg (Main body unit 430kg) Components: Main body unit (W1340×D825×H1740-mm) Controller (W236×D272×H121mm) Compressor (W300×D382×H334mm) Power: 1.5kw (Main body unit 1.1kw) Operating Voltage: AC 100V	tissues for applications in therapeutic development, regenerative medicine, and other areas in the life sciences Development of 3D-structures from cells only It provides the only-one technology for researchers	https://www.cyfusebio.com/en/
6	Digilab	Cell Jet 3D printer	The 3D-printed cells can be used for stem cell research, cancer biology, automated cell arrays, cell-cell or cell-drug interaction studies, tissue engineering or regenerative medicine.	Dynamic Dispense Range: 20nL to 1mL (with 190um ceramic tip, 1mL syringe and 1000ul loop) Dispense Speed: 30 seconds to dispense to a 96-well plate with a 1-channel system Dispense, Positioning Performance: Stepper Motor Resolution — 1.3um Repeatability < +10um	Has the unique capacity to print cells with 95 percent viability.	http://www.digilabglobal.com/products-celljet
7	Bio3D	SYN [^] and Explorer	The Bio3D SYN [^] supports a variety of different biomaterials and non-biomaterials all together in one single 3D bio print. Those 3D printing biomaterials include: whole cells, bacteria,	Technology: syringe-based extrusion/Materials: polymers, hydrogels Max. print speed: 0.47 mm/s Min. layer thickness : 0.01 mm	Bio3D produces systems which are not modified or ported from existing industrial or consumer 3D printers. Their 3D bio printers are specially designed and	https://www.antiwaa.com/product/3d-printers/bio3d-syn/

Table 4 (continued)

S. no.	Company	Name of 3D printer	Specialization	Specification	Remark	Reference (web link)
8	Advanced solution life science	BioAssemblyBot	proteins, biogels, polymers, food materials. BioAssemblyBot precisely builds 3D tissue structures and models, automatically switching between up to 8 independent syringe barrels.	Technology: six-axes syringe-based extrusion Materials: ND Dimensions 12" W x 20" H x 22" D 300mm x 520mm x 550mm Print Volume 176 in cubed 11,340mm cubed Resolution Z/R Linear Axis: 40 µin 10 µm Θ Rotary Axis: 40 µin 10 µm	built for scientific and research applications. listing all of its possible applications for cell systems and assays, experimental tissue models, organ models and microfluidic platforms.	https://www.advancedsolutions.com/bioassemblybot
9	nScript	1.3Dn Series 2.3Dn-DDM Series	nscript provides industrial precision microdispensing and direct digital manufacturing equipment with unmatched precision and quality	XY accuracy:0.015 mm Min. layer thickness:0.005 mm Weight:2722 kg Power input:120 VAC 20 A	can not only print out living cells but also extracellular matrices, collagen, hyaluronic acid and many more, while it creates Bioscaffolds such as biopolymers in any shape.	https://www.nscript.com/products/
10	EnvisionTEC	3D-Bioplotter	Which is an open-source materials printer enabling researchers to produce customized and patient-specific silicones, thermoplastics, ceramic and metal pastes, hydrogels, often alongside living cells	Footprint (L x W x H): 976 x 623 x 773 mm (38.4" x 24.5" x 30.4") Weight: 90-130 kg (depending on the model) Electrical Requirements: 100-240 V AC, 50/60 Hz Compressed Air Requirements: 6 - 10 bar (85 - 145 psi) Technology: syringe-based extrusion Materials: hydrogels, silicone, hydroxyapatite, titanium, chitosan	The 3D Bioplotter is used by researchers for fabricating scaffolds using the widest range of materials, from soft hydrogels over polymer melts up to hard ceramics and metals. This machine is specially designed for work in sterile environments in a laminar flowbox, a requirement of biofabrication, for example, when using alginate cell	https://envisiontec.com/3d-printers/3d-bioplotter/

Table 4 (continued)

S. no.	Company	Name of 3D printer	Specialization	Specification	Remark	Reference (web link)
11	3Dynamic Systems	Alpha & Omega	The 3Dynamic Alpha Series is a single extrusion bone tissue fabrication platform. The deposited material can be seeded with platelet-derived growth factor to create the right environment for tissue regeneration by recruiting stem cells that can produce bone and form a supportive structure, including blood vessels. The Omega Tissue Engineering Workstation is a dual extrusion 3D bioprinter used to generate heterogeneous tissues with a printable bioactive gel, protein growth factors, and scaffolds that mature into living tissue structures.	XY accuracy: 0.75 mm Max. build size: 150 × 150 × 60 mm Dimensions: 500 × 500 × 500 mm Power input: 1.5A 240V	suspensions for scaffold construction. 3D transplantable bone and complex tissue constructs on demand and successfully engineered a suitable bone composite and a 3D bioprinting technology to make high complexity tissue structures.	https://www.aniwaa.com/product/3d-printers/3dynamic-systems-alpha-series/
12	Nano3D Sciences(-n3D)	Nano 3D print	Using their Nano Shuttle technology, within a machine they call the Bio-Assembler, Nano3D is able to create 3-dimensional breast cancer tissue via the manipulation of cells using magnetic nanoparticles	Stage Dimensions: 214x186x160 (mm) / 8.4x7.3x6.3 (in) Stage Material: Removable glass heated bed Printing Layer Height: ≥ 0.02mm Positional Accuracy: XY axis: 0.011mm Z axis: 0.0025mm Nozzle Diameter: 0.4mm Max. Nozzle Flow Rate: 24cc/hour	they can either levitate or bioprint cells; and these cultures are faster to assemble than another system. Nano Shuttle, is able to create a plate of spheroids in 15 minutes to a few hours for medical research.	https://3dprint.com/22681/nano3d-rbcc-3d-print-cancer/

Table 4 (continued)

S. no.	Company	Name of 3D printer	Specialization	Specification	Remark	Reference (web link)
13	<u>Rokit</u>	Rokit In vivo 3D bioprinter	In vivo can print with a multitude of materials including PLGA, PCL, PLLA, collagen, alginate, and silk fibroin	<p>Max. Axis Moving Speed: 350mm/s</p> <p>Max. Nozzle Temp.: 270 °C</p> <p>Max. Heat Bed Temp.: 100 °C</p> <p>Machine Dimensions: 467x357x374mm</p> <p>Machine Weight: 13 kg (28.66 lbs)</p> <p>Printing system: polymer extruder+ bio dispenser</p> <p>Resolution: extruder 0.2mm, dispenser 0.08mm</p> <p>Build volume: 100x100x80mm</p> <p>Printing speed: 3 to 20mm/sec</p>	multi-use hybrid bioprinter with both an <u>extruder and a liquid dispenser</u>	https://www.aniwaa.com/product/3d-printers/rokit-3dison-invivo/
14	<u>GeSim</u>	<u>Bioscaffolder 2.1</u>	Tissue engineering and tissue regeneration becomes a promising approach e.g. to cure severe bone injuries. Artificial tissue grown from differentiable cells often needs to get in a particular 3-dimensional shape for implantation. Bio scaffolds can serve as a cell growth environment for artificial tissues, further supporting supply of the cells and removal of the metabolite	<p>Technology: syringe based extrusion and piezoelectric nano liter pipetting</p> <p>Materials: polymers, high viscosity paste materials, alginate, calcium phosphate, silicon, cells and protein solutions</p> <p>Power input: 115-240V</p> <p>ACDimensions: 975.36 × 622.3 × 772.16 mm</p> <p>38.4 × 24.5 × 30.4 in</p>	The Bio Scaffolder 2.1 is a modular instrument platform with up to four independent z-axes for running several dispensing tools. It can be adapted to your application as well as to your budget. It provides pressure-driven 3D printing as well as piezoelectric Nanolitre pipetting on the same instrument.	https://www.medicalexpo.com/prod/gesim/product-68587-710354.html
15	<u>REGEMAT 3D</u>	V1 Bioprinter	This 3D bioprinter is adaptable to different types of tissues, to research the biggest unsolved biotechnology problems and to battle	<p>Head technologies IPF, IVF, FDM microextrusion</p> <p>Number of biomaterials 2 for FDM, up to 8 for IPF/IVF</p> <p>Nozzle diameter</p>	The REGEMAT3D is a bioprinting modular system that integrates the latest advances in 3D printing and regenerative therapies to offer adapted	https://www.aniwaa.com/product/3d-printers/regemat3d-v1-bioprinter/

Table 4 (continued)

S. no.	Company	Name of 3D printer	Specialization	Specification	Remark	Reference (web link)
16	Allevi	Allevi 1 Allevi 2 Allevi 3	different kinds of pathologies, such as; Cardiovascular Disease (CVD), Peripheral Vascular Disease (PVD), Non-healing wounds, Spinal cord injury, Alzheimer's Disease (AD), Parkinson's Disease (PD), Musculoskeletal Disorders (MSDs), Autoimmune Disorders, Stroke, Diabetes, and Ocular Disease.	0.1–0.5mm Build volume 150mmx150mmx110mm X axis resolution 150 µm Y axis resolution 150 µm Z axis resolution:400 nm	solutions to fulfil research's needs	https://www.biotronhealthcare.com/allevi-1-bioprinter-2/
17	Poietis	NGB-R Bioprinter	With the smallest footprint and widest material capabilities of any 3D bioprinter on the market, It develops and produces human, customized tissues using 4D bioprinting for pharmaceutical and cosmetic applications as well as regenerative medicine	•11.8 × 11.2 × 10.8 in •Dimensions Metric (W × H × D)30.0 × 28.3 × 27.5 cm •Weight15.5 lbs (7.0 kg) •Power Requirements AC 110V – 220V Cell resolution 1 to 100 cells per droplet Printing speed 10,000 droplets per sec Precision 10 µm Droplet volume from pL to nL Viscosity 20 to 300 mPa.s min. Cell concentration up to 100 millions per ml Min. bioink volume from 4µL	With the Allevi 1 bioprinter, you can choose to cure biomaterials in either visible or ultraviolet light Combining laser-assisted, microvalve and extrusion bioprinting, it enables true versatility of bioprinting (from cell to spheroids) and offers the possibility of using a large number of biomaterials and hydrogels.	https://poietis.com/en/poieskin/welcome.php#poieskin1
18	RegenHU	3DDISCOVERY	For the driving force of scientific advancements in Tissue Engineering, Regenerative Medicine and Drug Discovery.	Work range:130x90x60 Precision:5 micrometer Modular printhead concept: General Temperature control: 0 to 80 degree C overall dimension: 600x700x670 mm	It provides two <u>cutting-edge bioprinting solutions</u> that work by combining multiple extracellular matrix materials, hydrogels, cells, and bioactives	https://www.regenhu.com/3d-bioprinters#3ddiscovery-evolution

Table 4 (continued)

S. no.	Company	Name of 3D printer	Specialization	Specification	Remark	Reference (web link)
19	3D Bioprinting Solutions	FABION	Tissue spheroids -- cell aggregates. Depending on the type of cells, of which it is built, a spheroid will have certain properties. For example, if fabricated of endothelial cells, spheroids with lumens will be formed characteristic of the blood circulatory system.	Technology: multiple (photocuring, electromagnetic and extrusion) Materials: hydrogel, organoids	within a three-dimensional spatially-controlled environment, enabling highly dynamic networks of proteins and signal transduction pathways in tissues, cell-cell and cell-extracellular matrix interactions. Bioprinter applies a layer of hydrogel (biopapers) in which tissue spheroids (bioink) are accommodated. The procedure is repeated as programmed to form layers, which may differ from each other, until the final layer is printed. This procedure allows creating a construct with a complex structure	https://bioprinting.ru/en/products-services/fabion/
20	JEMMA REDMOND	Ourobotics Revolution 3D bioprinter	Applications range from Human tissue, Pharmaceuticals, Food, Synthetic Biology, Electronics, Batteries, and even Textiles as the machine can print with just about every gel-like substance including Collagen, Gelatine, Alginates, Chitosan and more.	Technology: syringe based; syringe-based extrusion Materials: bone tissue from PCL, PLA, PGA, PEG, fibrin elastin, collagen, calcium phosphate and hydrogel	Actually, producing functional, replacement tissues and organs. In other words, creating (or extending) life itself.	https://3dprintingindustry.com/news/jemma-redmonds-ourobotics-low-cost-3d-bioprinter-does-10-materials-and-more-63237/

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