3D Bioprinting of Human Hollow Organs

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Abstract

3D bioprinting is a rapidly evolving technique that has been found to have extensive applications in disease research, tissue engineering, and regenerative medicine. 3D bioprinting might be a solution to global organ shortages and the growing aversion to testing cell patterning for novel tissue fabrication and building superior disease models. It has the unrivaled capability of layer-by-layer deposition using different types of biomaterials, stem cells, and biomolecules with a perfectly regulated spatial distribution. The tissue regeneration of hollow organs has always been a challenge for medical science because of the complexities of their cell structures. In this mini review, we will address the status of the science behind tissue engineering and 3D bioprinting of epithelialized tubular hollow organs. This review will also cover the current challenges and prospects, as well as the application of these complicated 3D-printed organs.

KEY WORDS 3D bioprinting · bioinks · biomaterials · hollow organs

INTRODUCTION

Printing technology has advanced over the past few decades from the age-old 2D printing to a complex successive layer-by-layer additive process of printing, also known as 3D printing (1, 2). The 3D structures formed with this complex geometry by printing materials layer-by-layer have opened doors of innovation and personalized product manufacturing companies. In addition to these applications, the 3D printing sector has also developed a major footing in medical science. Our body has a unique ability for regeneration, but nevertheless, it is constrained by various factors such as growth hormones, differentiation, tissue type, and physical size. If there is damage done to any human wound, the tissue may require external support (scaffolds) to regenerate itself. These scaffolds serve as a platform for cells to move to action sites and reformulate new tissues. Scaffold designs play an indispensable role in tissue regeneration in this 3D bioprinting technology (3, 4). Researchers and scientists may now use 3D printing to develop, envision, handle, and test their ideas in real space.

The term "3D printing" was first enacted by Charles W. Hull in 1986. He named the method "stereolithography," where he used a thin layer of materials sequentially printed layer by layer with UV light to form a 3D structure (5). This method was later modified using biomaterials to form 3D-printed scaffolds of cells. The next advancement was made possible by the latest tissue regeneration 3D printing technique using cellular biology, also known as "FRESH," or "Freeform Reversible Embedding of Suspended Hydrogels," discovered by Professor Adam Feinberg and his team from Carnegie Mellon University. This technique was used to print more complex organ structures such as the heart and lungs, printing soft biomaterials within a gelatin bag to overcome the printing constraints of soft and low viscosity bioinks (6).

3D bioprinting involves layer-by-layer exact positioning of biological materials, bioinks, and live scaffolds, as well as spatial control of functional component placement, thereby making this one of the most complex processes. There are several bioprinting techniques and approaches by which researchers are fabricating functional 3D-printed human organ constructs.

Biomaterials are natural or synthetic substances containing living stem cells, which are also called bioinks and are

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the key components of bioprinting. Any tissue or organ of the human body at any time can be restored, reinstated, or augmented when bioinks meet the biological systems (7).

3D bioprinting is used for developing *in vitro* models for testing pharmaceutical drugs on animals, which has the potential to minimize these lengthy and expensive clinical trials and save billions of dollars for pharmaceutical companies. Bioprinting can save lives by providing patients with personalized organs made from their cells, lowering the risk of organ rejection. All that is required of patients is for them to wait for the organ or tissue to be printed!

In this review, we will discuss the techniques and approaches of bioprinting. We will discuss how various human body hollow organs can be 3D printed and what the major limitations are that are faced. Finally, we will discuss the various bioinks used for the preparation of modern biomaterials and the challenges in future research.

BIOPRINTING TECHNIQUES AND APPROACHES

3D bioprinting of tissue is a six-step process, as shown in Fig. 1. The design of bioprinted tissues can be guided by imaging of the injured tissue and its surroundings. Design techniques employed alone and in combination include biomimicry, autonomous self-assembly tissue, and mini tissue building blocks, which will be discussed later. The selection of materials and cell sources is critical to the tissue's structure and function. Synthetic or natural polymers, as well as decellularized extracellular matrices, are common materials. Allogeneic or autologous cell sources are both possible (8). These components must work with inkjet, micro extrusion, and laser-assisted printers, among other bioprinting systems. Some tissues may need to

mature in a bioreactor before being transplanted. Finally, these 3D-printed tissues are used in implantation and *in vitro* testing applications.

The design approach techniques are as follows.

Biomimicry This is the process of creating exact replicas of a tissue or organ's cellular and extracellular components through 3D bioprinting (9). This can be accomplished by replicating certain cellular functional components of tissues, such as duplicating vascular tree branching patterns or creating physiologically appropriate biomaterial varieties and gradients. The success rate of this approach depends on the micro-scaling capabilities of the given biological tissue.

Autonomous self-assembly tissue Usually, the use of embryos for organ development is another approach to biological tissue replication. This "scaffold-free" variant goes through cellular organization and fusion to resemble the developing tissue.

Mini tissues This concept applies to both the technologies mentioned above. This process resembles assembling both the above strategies to form a large construct. 3D bioprinting precisely duplicates functioning tissue units to form "organs-on-a-chip," which are maintained and interconnected by a microfluidic network for use in drug and vaccination screening, as well as *in vitro* disease models (10–12).

The significant progress in this printing technology has resulted in more than forty different 3D printing processes. Various techniques like inkjet bioprinting, extrusion printing, laser-assisted printing, and stereolithography are the most generally accepted technologies in bioprinting. The comparison of the various techniques is shown in Table I and Fig. 2, with each technique being discussed in further detail below.
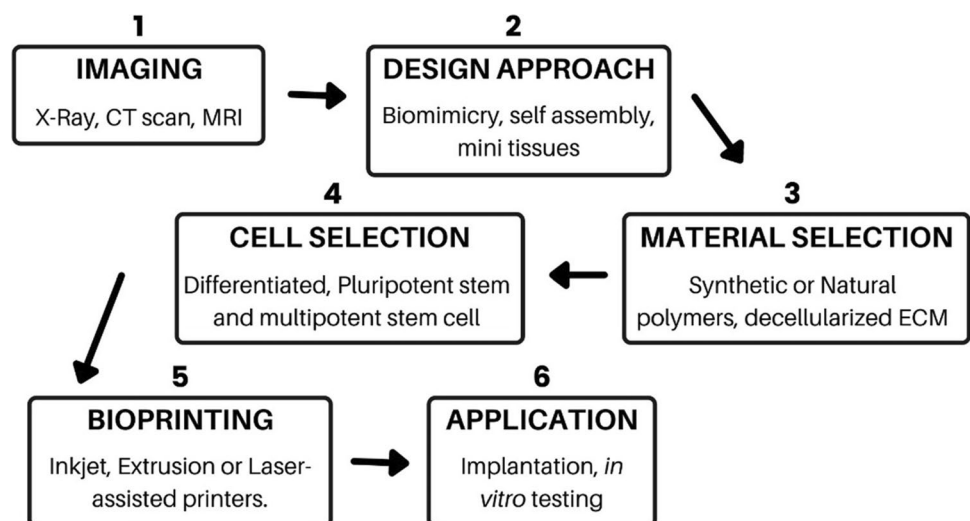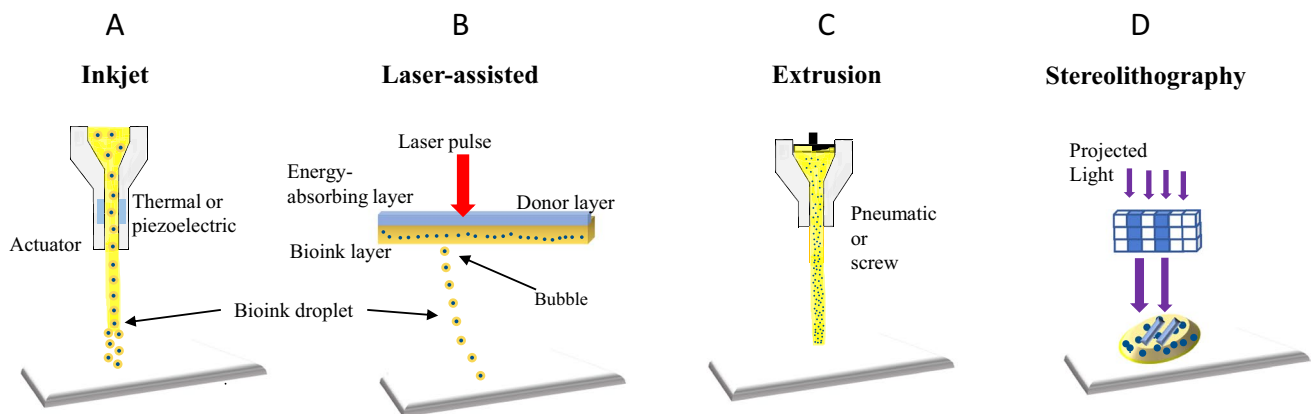
Fig. 1 Steps of 3D bioprinting

Table I A Comparison of Different Bioprinting Techniques

	Inkjet printing	Extrusion	Laser-assisted	Stereolithography
Droplet size	50–300 µm	5 µm to 1 mm	> 20–80 µm	N.A
Print speed	Fast	Slow	Medium	Fast
Cell density	Low	High	Medium	Medium
Cell viability	> 85%	As low as 40%	> 95%	> 90%
Printer cost	Low	Medium	High	Medium
Resolution	50 µm	100 µm	10 µm	100 µm
Advantages (9, 10, 22)	High speed, availability, low cost	Ability to use high viscosity bioink and print high cell density	A high degree of precision and resolution ability to use high viscosity bioink and print high cell density	A high degree of fabrication accuracy and low printing time
Disadvantages (9, 10, 22)	Lack of precision in droplet placement and size, need for low viscosity bioink	Distortion of cell structure	Time-consuming, high cost	Use of high-intensity UV light, lengthy post-processing, lack of compatibility materials

Fig. 2 Simplified illustrations of 3D bioprinting types: **A** inkjet bioprinting, **B** laser-assisted printing, **C** extrusion bioprinting, and **D** Stereolithographic printing. Recreated from Mahfouzi *et al.* (29)

Inkjet Bioprinting

In the 1970s, Hewlett-Packard Company invented inkjet printing as a 2D printing technique. Later, inkjet printers were altered with a chamber and a conveyor phase control for the Z-axis for the printer, which was considered to be one of the first 3D printing techniques (13). Inkjet printers are also known as "drop-on-demand printers" (14) as these printers can regulate the size of the printed pattern and the deposition of generated droplets with the help of a digital controller on a computer (15, 16). Nowadays, there are four approaches to inkjet droplet squeezing: thermal, piezoelectric, acoustic, and electrostatic inkjet printing. The most commonly used materials for structure construction are thermal and piezoelectric (17). In the context of bioprinting, these materials help to expel successive drops of bioink onto a substrate, thereby helping to replicate a computer-aided drug design with printed tissue (13).

Extrusion Bioprinting

Extrusion bioprinting is the latest version of inkjet bioprinting. In this process, low viscosity bioink is used in inkjet bioprinting to be ejected through the generated air bubbles. Extrusion bioprinting uses mechanical and pneumatic screw plungers to expel the bioinks. Except for foamy droplets, randomly applied force creates continuous cylindrical lines. Different types of viscosities, mainly highly viscous bioinks, are used in extrusion bioprinting. Computer-aided operators regulate the extrusion procedure, speed, and deposit location as per flexibility (18). This system is also known as a "direct writing system." Here, materials are ejected out of the container as continuous filament (19), where various objects such as polymers, cells, and cell capsulated matrix can be utilized in bioprinting (20, 21).

Laser-assisted Printing

More than three decades ago, Bohandy *et al.* introduced the laser-induced forward transfer bioprinting technique (22). This technique permits the high-resolution deposition of material in liquid or solid phases (23). The photo-polymerization method can modulate laser-assisted bioprinting of biomaterials to print a wide variety of cells without affecting cell viability (24). This system consists of four components: a receiving substrate, a metallic ribbon film that absorbs the laser energy, a pulsed laser source, and a laser focusing instrument (25). The structure ribbon is made up of two layers, with the upper absorbing energy layer being mostly glass covered with a sub-micron titanium and gold film. Continuation of the production process: the laser pulse concentrates on the designed area with the help of film evaporation on the upper layer. At the bottom layer, a high-pressure bubble is formed at the interface with the suspended bioink. And then, the bioink is further ejected onto the receiving substrate in droplet form. The *z*-axis movement is maintained by an elevator system. Notably, this printing technology does not have any nozzles, yet can utilize many different kinds of materials, such as epoxide-based photoresist SU-8, ceramic materials, hydrogels, and cell-encapsulated materials (26).

Stereolithography

In the 1980s, stereolithography evolved as a solid-free fabrication technique. This process is closely related to the laser-assisted bioprinting technique: With the help of ultraviolet (UV), light stereolithography printing selectively hardens the photosensitive polymer layer-by-layer and ultimately produces a multiplex structure (27). First, a digital design resembling a structure is printed. The formatted structure of the organ in the stereolithography file is transformed from computed tomography (CT) scan images and magnetic resonance imaging (MRI) images (27). Next, computer-aided design (CAD) converts this structure, which is divided into slices and can be restored layer by layer. Another major factor in stereolithography is UV curing kinetics, which is a top-down printing process where the UV light thickens the lower layer and overlaps the layers to form the structure (28).

There are several other requirements for bioprinters to properly print lungs and alveoli, for example. They must support various types of multiple nozzles for printing different parts of the lungs at the same time, e.g., one of the nozzles will print supports for the structure of the lungs and the other nozzles will have different bioprinting types to print micro to nano alveoli resolution (29). Additionally, the printing speed must be optimal to protect both the cells and the bioinks while we are printing, as extended printing procedures may impair cell viability (30).

Overall, stereolithography offers the necessary high-resolution alveolar sacs for printing and creates abundant constructs with typical complex geometrical structures in a short amount of time, whereas other bioprinting processes, on the other hand, lack the requisite resolution or vertical printing quality for large structures. The pros and cons of 3D bioprinting are given in Table II.

PRINCIPLE OF BIOPRINTING HUMAN ORGANS

3D bioprinting is a cutting-edge technique that uses additive manufacturing technology to create live tissues such as blood vessels, body organs, and skin (31). The goal of 3D bioprinting is based on the precise placement of biological

Table II Advantages and Disadvantages of 3D Bioprinting

Advantage	Disadvantage
1. 3D printing is a quick process to manufacture parts that allow moderation of each design to be completed faster such as injection molding	1. During the 3D printing process, parts are created layer by layer. When the layers separate under stress, the part structure can break down
2. 3D printing assures the quality of products by their remarkable designs more so than the traditional process which has poor designs that result in low-quality products	2. 3D printing is not flexible to work with most raw materials because most of the printable 3D materials cannot be recycled
3. 3D printing saves transportation and import costs compared to traditional processes	3. 3D printing consumes high energy to produce large quantities. Hence, it is most suitable for only small quantity production
4. Lightweight materials used in 3D printing are plastic, which makes them much lighter than their metal counterparts (8)	4. Highly volatile organic compounds emitted by 3D printers are carcinogenic and toxic and can cause serious health problems like organ damage, throat irritation, and nausea (29)
5. 3D printing makes more flexible or free designs that help to create any type of geometry	5. 3D printing machines and materials are more expensive than traditional equipment
6. 3D printing technology reduces the waste of materials than the traditional process	6. Many 3D-printed products need post-processing which depends on several factors such as the size of the part and the application of finished products

elements, biochemicals, and living cells, including bioinks in the form of a layer-by-layer structure through a bio-printer. The main applications of 3D bioprinting are shown in Fig. 3.

The principle of bioprinting is generally based on biopolymers and stem cells that are mostly used as bioinks (same as ink for any printer), which are filled into the 3D printer. The 3D printer then uses the ink to print a 3D organ (output from the printer). 3D organs are prototypically similar to real organs. These 3D-printed organs can be used *in vivo* as organ transplants or *in vitro* for clinical trials of new drugs.

3D Printing Software Applications

A 3D model created in a modeling application is the starting point for every 3D print. 3D modeling software allows the user to build the prototype from scratch virtually, thereby saving time and money. A doctorate professor at the Massachusetts Institute of Technology (MIT) is credited with developing computer-aided design (CAD), and the first-ever CAD software was called Sketchpad. These breakthroughs lay the groundwork for today's CAD technology, which allows 3D bioprinting to become a reality (27). CAD helps to build the 3D-printed organ to fit perfectly according to the patient's needs. The first effective research outcome utilized the use of CAD technology to bio print 3D scaffolds in the early 1990s. CELLINK created its own software systems called HeartOS, DNA Cloud, and DNA Studio to handle the bioprinting process. TinkerCAD is mostly used for modeling basic geometry or manipulating meshes that already exist. Furthermore, additional specialist software tools such as Meshmixer may be required to

post-process models produced using TinkerCAD in some circumstances (28).

3D BIOPRINTING OF HOLLOW TISSUE OR ORGANS

3D bioprinting would be a solution to global organ shortages and the growing aversion to testing new cosmetics, chemicals, and pharmaceuticals on animals. After the discovery of the traditional 3D printing technique that prints using a layering process using source material such as plastic, to a full-form object, 3D bioprinting organs became a much more complicated process.

Airways

Lungs

The fourth greatest cause of mortality globally is an end-stage lung disease, often known as a chronic obstructive pulmonary disease (COPD) (32). As rates of tobacco smoking and exposure to air pollution are rising, the number of COPD patients will rise even faster, prompting the need for immediate development and innovation of novel treatment options. In this circumstance, extracorporeal membrane oxygenation (ECMO) and mechanical ventilation can be used as a bridge to lung transplantation, which is still the only definitive therapy, but the requirement for immune suppression and the scarcity of donor organs are substantial roadblocks to a wider therapeutic effect (33). Potential developments in tissue bioengineering, on the other hand, may be able to circumvent the lack of donor organs and the necessity for immune suppression, organ shortages, and rejection of organs. Clinical research claims that there are many chronic consequences of the COVID-19 virus with lungs, heart, and/or kidney damage (34, 35). SARS-CoV-2 in contact with our body leads to increased levels of pro-inflammatory cytokines, thereby showing pulmonary fibrosis and uncontrollable lung inflammation as common pathological signs (36, 37). To develop successful therapies, researchers must first identify the fundamental immunopathology and inflammatory response of the SARS-CoV-2 infection, which is a significant clinical research challenge. Therefore, there is a recent urge to prepare a model for 3D *in vitro* lungs for evaluation of the trajectory of the COVID-19 virus and the therapeutic efficacy of drugs for the same.

Lung cells are known to have responsive regenerative capabilities to selective stimuli and injuries. They react to stress by reactivating the cell growth cycle to repair the damaged cell. Type II alveolar cells are ideal candidates for potential 3D bioprinting-based testing and therapy, as they show proliferation to form Type I alveolar epithelial
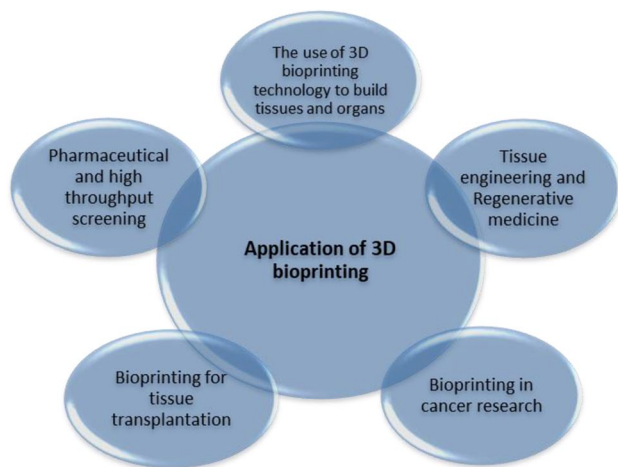
Fig. 3 Application of 3D bioprinting

cells (38). These Type II alveolar variant stem cells can be collected and can be printed *in vitro*, which is known as broncho-alveolar stem cells (39).

3D bioprinting may create functional tissue that looks and functions like natural tissue, but it must meet specific criteria in terms of material properties and geometrical scaffold design. The synthetic scaffolds for bioprinting lungs are to be made biocompatible, non-immunogenic, non-toxic, as well as chemically stable, and they should not show an adverse immune response after host implantation (38). The ideal candidates for reproducing complex lung structures are mostly synthetic hydrogels such as polyvinyl alcohol (PVA), polyglycerol sebacate (PGS), and polyethylene glycol (PEG) (40–42). Collagen, Matrigel®, Gel-foam, Polyglycolic acid, and PluronicTM F-1379 are the most commonly used natural polymers for lung tissue regeneration (43). The fabrication of a 3D collagen scaffold is done using biomimetic collagen with collagen-binding hepatocyte growth for alveolar regeneration after acute lung injury (44). For human lung epithelial cells, synthetic polymers such as PDLLA (poly (D, L-lactic acid)) are used, which can provide a biocompatible environment for pneumocytes (45). For the preparation of polymer-based 3D bioprinting of tracheal grafts, synthetic polymers like polycaprolactone (PCL) and polylactic acid (PLA) are used, in addition to natural polymers such as collagen, fibrin, alginate, or even decellularized extracellular matrix (dECM) (46). A brief table on the chemical and biological significance of the polymers used in 3D bioprinting is given in Table III below.

The lungs are made up of many branching airways of varying diameters, ranging from 1.5 cm at the trachea to 0.5 mm at the bronchioles (47). The conducting zone of the lungs is made up of vast airways that go from the major bronchi to the terminal bronchioles, which are not used for gas exchange. A pseudostratified epithelium lines the trachea and major airways, consisting of surface serous, goblet cells, submucosal mucous, submucosal serous, Clara, and brush cells that generate mucus and remove debris by ciliary activity (48, 49). The respiratory bronchioles are the smallest generations of airways leading to pulmonary acini, which are made up of numerous single alveoli and alveolar sacs (Fig. 4), which are regarded as the respiratory zone of the lungs or gas-exchanging sites. Human lungs constantly breathe to take oxygen for vital capacity and evacuate carbon dioxide, which is generated as a by-product. When oxygen enters the body, it reaches the alveoli through the airways and is replaced with carbon dioxide carried by the blood through the capillaries of the alveoli (50). The lungs have two sections: One is the airway, and the other is the vasculature. Airways are the main part of the pulmonary system. A large airway is called the conducting zone of the lung, where no gas exchange takes place. The smallest airways are known as respiratory bronchioles, leading to the alveolar

sac and alveoli (51, 52). Moreover, the dual vasculature of the lungs adds much more complexity to the anatomy of the lungs.

The main role of the lungs is to take in oxygenated air from the atmosphere and pass oxygen into the bloodstream, which circulates to the rest of the body (53). The lungs are also important in the body's defense against infection and other harmful environmental factors.

The primary goal of pulmonary tissue engineering is to create a tissue that mimics natural lung tissue. To define the criteria for 3D bioprinting, a thorough understanding of scaffold characteristics and lung anatomy is required. Tissue engineering aims to reproduce the entire spectrum of specialized lung tissues and therefore offers physiologic capabilities via bioengineered air passages and vasculature gas exchange (33).

The major limitations for lung tissue regeneration are recreating the complex architecture of the lung, including a complex vascular flow network for gas exchange and blood flow.

In the early 21st century, researchers discovered that living stem cells from alveolar tissue could be sprayed through the nozzles without damaging the living cells. However, they need a favorable environment with food, water, and oxygen, which is nowadays provided by gelatin-enriched microgel (54). Due to their size and intricacy, bioprinting of alveoli remains a technological hurdle. This complex architecture of the vessels of the lungs is one of the unsolved questions that most researchers are focusing on today.

The shear pressures imparted to the cells as they travel through the needle restrict the resolution of current extrusion-based printers, which is about 100 μm (55). Stereolithography can generate high-resolution structures (up to 100 μm), but it is limited in terms of composition complexity since it typically uses only one material source. Single-cell precision with multiple cell types is possible with laser-induced forward transfer (10 μm) and some droplet-based printers (50 μm), but the current production speed is insufficient for producing significant clinical-sized tissues, and then further trying to incorporate them into the lung's hierarchical system would have been a challenging task (56).

Most of the alveolar model research primarily concentrates on replicating the air/fluid/cell interaction. Huh *et al.* (2010) used a poly (dimethylsiloxane) microdevice to build a breathing alveolar model, with this research area growing substantially due to "organ-on-a-chip" methods (10). Horvath *et al.* (2015) took this concept a step further by alternating printing of Matrigel® layers with both epithelial and endothelial cells across the porous membrane, which demonstrated better cell-cell interaction and cell coverage than other manual 3D printing techniques (57). The inability to print complicated vascular networks, such as airways and blood arteries in the lung and blood vessels in the liver,

Table III Polymers Used in 3D Organ Printing: Chemical and Biological Significance

| Polymers	Biological significance	Chemical significance	Limitation	Application
Alginate	Good biocompatibility including low toxicity, non-immunogenicity, rapid biodegradability, and chemical gelling	Alginic acid salts composed of β-d-mannuronic acid (M block) and α-l-glucuronic acid	Potential to cause stem cell death due to their extreme variance from a true physiological ECM	Vascular cartilage and bone tissue printing (40)
Polylactic acid (PLA)	Biodegradable, biocompatible, no toxic fumes	Aliphatic polyester formed from ring-opening polymerization of lactide or polycondensation of lactic acid monomer	Lowers glass transition temperature	Tracheal graft (41)
Polyhydroxy alkenoate (PHA)	High biodegradability, high biocompatibility, brittle, and tough nature	Thermoplastic polyester of hydroxy alkanoic acid	Nano thermal processing window	Fabrication of heart valve, bone scaffolds (42)
Polycaprolactone (PCL)	Biocompatible, non-toxic polymer	Semicrystalline and biodegradable polyester	Hydrophobic causes low bioactivity, slow cell growth tissue adhesion	Tracheal graft joint, cartilage, and trabecular bone (44)
Polyethylene glycol	Nonbiodegradable, poor mechanical strength	Hydrophilic polymer, linear, or branched structure contains asymmetric and dissymmetric hydroxyl ion as its tail group	Hydrolytic and enzymatic degradation easily degrades the PEG	Formation of keratin layers (45)
Polyether ketone (PEEK)	Superior biocompatibility, strength, and elasticity comparable to cortical bone used in prototyping craniofacial implants and bone refreshment	High performance, temperature resistance semicrystalline polymer, biologically inert, radiolucent	Bio-inertness causes reduced osteo-integrative properties, can catalyze reactions such as dislodging, encapsulation, and extrusion in the body	Prosthetics, artificial bone, heart and its parts, and other human parts (46)
Polyglycolic acid	Good biocompatibility, PGA biodegradation produces glycolic acid monomer, which is further metabolized to CO and water, both of which are nontoxic. The use of copolymer enhances their mechanical strength	Chemically versatile, linear polyester which upon degradation produces nontoxic metabolites	Susceptible to erosion resulting in scaffold collapse	3D scaffold architecture, used in bone internal fixation devices, preparation of resorbable sutures (46)
Polylactic co-glycolic acid (PLGA)	Cytocompatibility and biodegradable	Hydrophobic nature, linear structure	Hydrophobicity, usage limited to scaffold material, increase inflammatory reaction	Bone regeneration animal models and other tissue restoring systems (42)
Polyvinyl-alcohol	Biocompatible biodegradable, semicrystalline structure allows efficient oxygen and nutrient passage to cells	Bioinert, semicrystalline nature, hydrophilic, chemical stability in extreme pH and temperature	Hydrophilicity causes uncontrolled swelling	SLS bioprinting, bone cell ingrowth, used in craniofacial treatment, bone tissue engineering (46)
Polyurethane	Excellent biocompatibility and mechanical strength, good cytocompatibility	Multiblock polymers with either aromatic or aliphatic isocyanates	Poor thermal capability, poor weatherability	SLA and DLP printing technique, high printing resolution. Chondrocyte manufacture in cartilage tissue engineering, bone fabrication, construction of muscle and nerve scaffold (44)
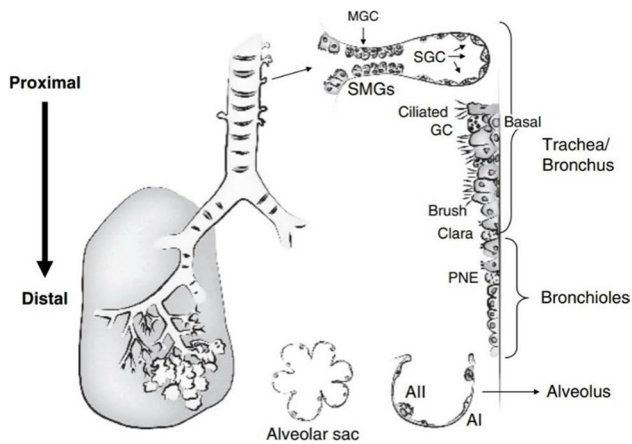
Fig. 4 Structure of lungs and various pulmonary cells along with their location; reprinted with permission from Chang *et al.* (49), Copyright Wiley 2008

Fig. 5 Printed hydrogel containing the lung subunit during RBC perfusion and ventilation of air sacs; reprinted with permission from Grigoryan *et al.* (54), Copyright 2019 Science by CC BY 4.0

has been one of the major hurdles in producing these tissue substitutes. However, Jordan Miller and his team have developed an open-source bioprinting technique known as a "stereolithography apparatus for tissue engineering" (SLATE). This method includes printing a structure layer by layer with a liquid pre-hydrogel solution that solidifies when exposed to blue light. The researchers used this technique to create an artificial lung-like structure replete with airways and blood arteries.

The first proof of the concept of having 3D-printed lungs (Fig. 5) was given by Jordan Miller and his team. His team, after several research trials, made an artificial version of the alveoli sac that has many independent vascular structures (54, 58). They built a "breathing model," including tidal air ventilation and blood flow, using poly (ethylene glycol) diacrylate and a stereolithographic printer. They were able to demonstrate pulmonary transport using this model by monitoring blood oxygenation entering and exiting it. Even though the printed vasculature was just 300 µm in diameter, it is nevertheless a significant step toward creating therapeutically useful lung tissues (54).

The 3D lung model recently made by a research team from POSTECH consists of four human alveolar cells, namely, lung fibroblasts (MRC5), lung microvascular endothelial cells (HULEC-5a), and type I and II alveolar cells (NCI-H1703 and NCI-H441), and was done using inkjet bioprinting techniques (50). Sungjune *et al.* (2021) (50) have made the 3D-printed alveoli from the thin layer of epithelial cells that are surrounded by capillaries that mimic the structure of hollow grapes. The alveolar membrane, which is a carrier of oxygen and carbon dioxide, is made of three layers consisting of epithelium, endothelium, and a basement membrane, as shown in Fig. 6. These layers are very thin to aid in gaseous exchange. Still now, precisely
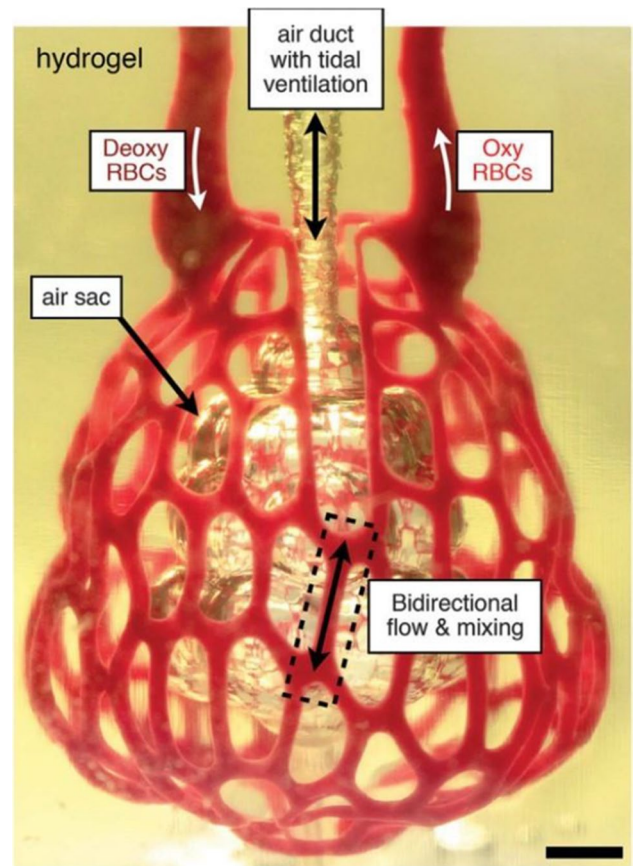
reconstructing alveoli with such a thin and intricate structure has been difficult. They have made this three-layered alveolar model with a thickness of 10 µm using high-resolution drop-on-demand inkjet printing. Drop-on-demand inkjet printing is a method that allows cells and biomaterials to be accurately printed in the desired place by releasing
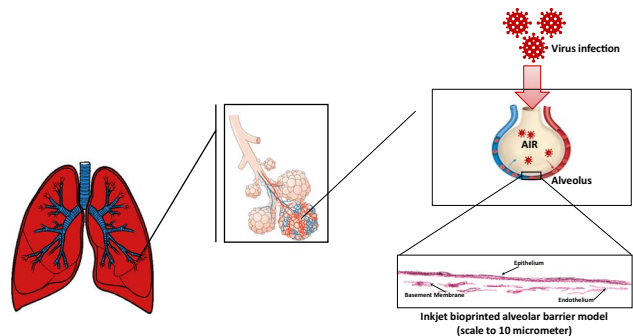

Fig. 6 The 3D bioprinted lungs' structure

ultra-fine ink droplets with the pressure delivered to the cartridge. Sungjune and his co-worker also used influenza virus infection on the model, and it responded like the physiological tissue and with similar regards to viral infection. They observed the self-proliferation and antiviral response to the influenza virus.

This artificially generated tissue can be an early platform for evaluating the therapeutic efficacy of various infected respiratory viruses, including the COVID-19 virus. It allows for mass manufacturing, quality control, and the creation of patient-specific disease models (50, 59).

Trachea

Environmental pollution, along with a bad and stressed lifestyle nowadays, causes many health problems in our body, including deadly diseases like cancer, especially tracheal tumors, which are the most prevalent. These tumors are malignant and cause narrowing of the trachea, thereby causing difficulty in breathing. Previously, various methods were used for treating this using tissue engineering or tissue grafting. Moreover, artificial implants were also attempted in an early stage, which failed as they were unstable and caused chronic immunosuppression (60). Although in the present day, despite the resolution of these main problems, there are still some immune reactions and dislodgement issues with tracheal implants. Various attempts to apply 3D bioprinting technology in tissue engineering have been made (20) using various biodegradable materials such as polycaprolactone (PCL), polylactic acid (PLA), and poly(lactic-co-glycolic) acid (PLGA) (61). Moreover, with the recent development of 3D printing technology, living stem cells or chondrocytes can be added to the hydrogel for the printing of the artificial trachea (62).

The trachea is the main airway that connects the larynx to the bronchi and allows air passage through the lungs. While the trachea is an airway, it differs from the lower airways of the lungs in terms of morphology. It is made up of 16 to 20 cartilage rings surrounded by connective tissue and smooth muscle that help to maintain the pressure due to breathing and prevent it from collapsing (63). Modern tracheal allograft fabrication techniques are significant antecedents to entire lung 3D bioprinting. In the following part, we will analyze the possibility of these developing technologies in future pulmonary bioprinting.

Dual head printing technology Kaye *et al.* (2019) first reported on this dual-headed printing technology, which was used for bioprinting alginate or collagen in partial ring design and polycaprolactone (PCL) (64). This experiment was tested on white rabbits in New Zealand, where the complete tracheal graft was implanted inside and was harvested at three or six weeks post-implantation (64). Although the

regenerated cartilage had a decent score when evaluated by the O'Driscoll score (65), tragically, all the grafts were severely stenosed.

Later, Bae *et al.* (2018), using the complex printing ability on larger geometry, produced a tracheal graft using alginate and PCL in an alternating overlapping pattern (illustration shown in Fig. 10D) (66). These grafts were also implanted on the same subjects as previously and observed for twelve weeks. This experiment turned out to be better than the previous one in that it had no complications or stenosis.

This experimental work was continued by Park *et al.* (2019), who continued the study of the graft up to 1 year after the implantation, where he found a few more complications, which are now being studied as the further development of 3D bioprinting of the trachea continues (67).

Organoid printing technology Taniguchi *et al.* (2018) introduced a new method, shifting from using the traditional polymer approach to using a removable needle. The endothelial cells, chondrocytes, and mesenchymal stem cells were isolated and cultured from F33 rats, which were then aggregated together (68). These were assembled into a tracheal construct using a needle in between. After one week, these needles are removed and replaced by a stent, which is then kept in the bioreactor for four weeks. This tracheal construct is then implanted inside a rat and observed for 23 days (illustration is shown in Fig. 10E).

Later, this method was modified by Machino *et al.* (2019), where the elastic tissue was replaced by more realistic native tracheal cells (69). Silicon stents were also used in place of plastic stents. This method was perfectly suitable for broader airways but faced challenges for smaller airways and alveoli. These challenges are the major focus of recent studies.

In addition to the above-mentioned bioprinting methodologies, there are a variety of tracheal building procedures that rely on 3D-printed plastic scaffolds that might be transferred to bioprinting and whose results would be useful to the future growth of bioprinting.

Alveoli

Alveoli are the endpoint and smallest structures in the respiratory system. They have a small balloon-like structure with a high surface area and a thin wall for the exchange of gases between the alveoli and small blood vessels known as capillaries. Mostly, they form a cluster and are arranged throughout the lungs.

Considering their size and intricacy, bioprinting of the alveoli remains a technological hurdle. The shear pressures given to the cells as they travel through the needle restrict the resolution of current extrusion-based printers, which is roughly 100 μm (55). Stereolithography, laser-induced, and

some other droplet-based printers were unable to print such complex structures with high resolution and with the precision of single or multiple cells. So, at the 3D alveolar resolution, there has been a minimal success in entirely recreating the alveolar barrier.

Huh *et al.* (2010) pioneered the use of poly(dimethylsiloxane) to create a breathing live model of alveoli (10). Horvath *et al.* (2015) continued this alternating printing approach by using Matrigel® to keep epithelial and endothelial cells over the porous membrane (57). Later, Grigoryan *et al.* (2019) started working on a more complex structure of the lung alveoli model and made a lung model that had vascular structure and airway spaces (Fig. 5).

Cardiac Tissue

The heart is a big muscular pump that is split into two parts, one on the right-hand side and the other on the left-hand side, which is further divided into two parts, thereby making four chambers (Table IV). The right-hand side of the heart is responsible for the pumping of deoxygenated blood to the lungs. The left-hand side pumps oxygenated blood throughout the body.

The left ventricle's (also known as the strongest chamber) vigorous contractions are the cause of our high blood pressure. The coronary arteries run along the surface of the heart and provide oxygenated blood to the heart muscle. A web of nerve tissue also runs through the heart, directing the complex signals of contraction and relaxation. The sac that surrounds the heart is called the pericardium (Fig. 7).

In today's world, heart and cardiovascular disease are major health concerns. Heart transplantation is currently the only good option for people with severe heart failure. In the case of heart failure, allografting or organ transplantation has been used, but it has a limit due to organ unavailability and immune rejection (70, 71). Hence, heart tissue regeneration is a new and innovative approach for repairing the heart valve and cardiac damage (concept illustration shown in Fig. 8, 3D-printed heart shown in Fig. 9). To study heart valve interstitial cell response, the most used bioink is cell-laden polyethylene glycol-based hydrogels (72, 73). Hydrogels are hydrated networks of cross-linked polymers when a hydrogel forms gelation cells that can be enclosed in 3D bioprinting (74). Natural polymers such as agarose, alginate, collagen, chitosan, and gelatin, as well as synthetic
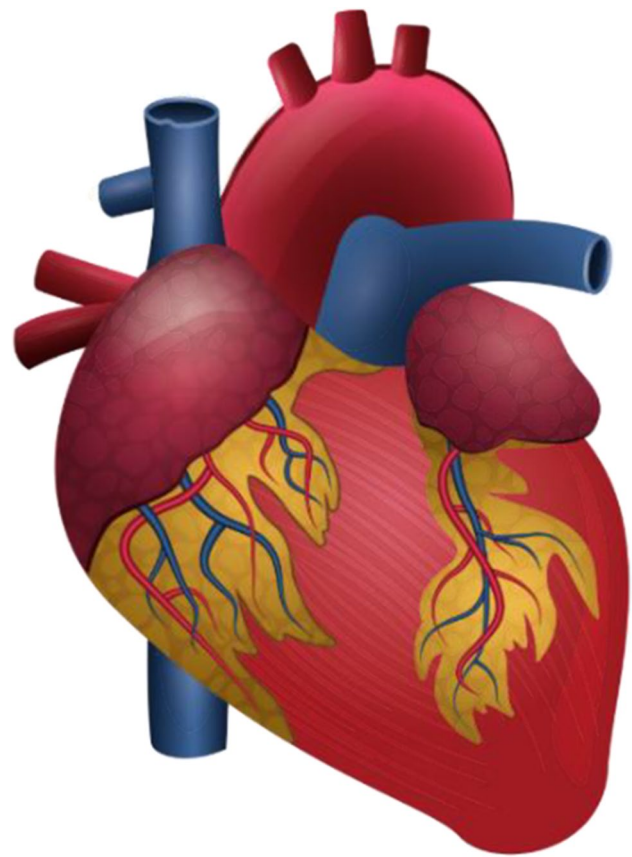

Fig. 7 Human heart structure

polymers such as polyethylene glycol and pluronic acid, or a combination of both, can be used to make these cell-laden hydrogels. Heart cells have low cytotoxicity and structural similarity to the natural extracellular matrix (ECM), which is an important component of the heart cells being incorporated into 3D bioprints. Another bioink contains different types of proteins, glycoproteins, and proteoglycans, which are the decellularization of syngeneic tissue (75).

Tissue regeneration is a technique that forms 3D structures and assists in computer-aided design (CAD). It was discovered that the function of the ventricular heart may be improved by substituting necrotic tissue with tissue-engineered constructs known as cardiac patches, which perfectly match the patient's biochemical, cellular, immunological, and anatomical qualities. Synthetic polymers, hydrogels, and

	Chamber	Function
Table IV Chamber of Human Heart	Right atrium	Receives blood from the whole body and pumps it to the right ventricle
	Right ventricle	Receives blood from the right atrium and pumps it to the lungs
	Left atrium	Receives oxygenated blood from the lungs and pumps it to the left ventricle
	Left ventricle	Pumps oxygen-rich blood to the rest of the body

Fig. 8 Schematic concept diagram of 3D bioprinting of the heart. The patient's omentum tissue is taken, and the cells are isolated from the extracellular matrix, before being processed into a customized thermosensitive hydrogel. The pluripotent reprogrammed cells are then differentiated into endothelial and cardiomyocyte cells before being encapsulated in a hydrogel to produce the bioinks used in 3D printing. The bioinks are then used to create vascularized patches and complicated cellularized structures, which are subsequently printed. The autologous designed tissue that emerged can be implanted back into the patient to replace or repair diseased or damaged organs with minimal risk of rejection. Reprinted with permission from Noor *et al.* (88); Copyright Wiley 2019
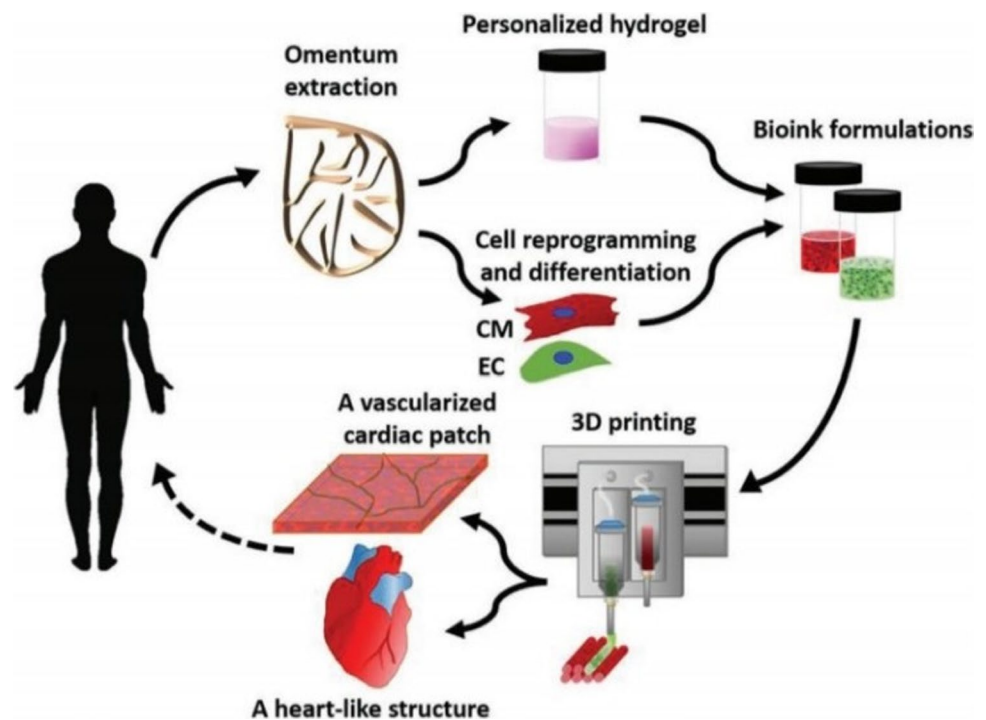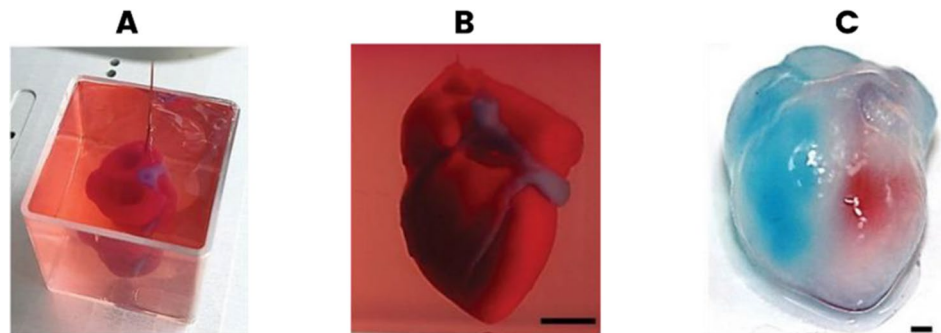

Fig. 9 A 3D-printed heart. **B** Freshly printed heart. **C** Post extraction heart; reprinted with permission from Noor *et al.* (88); Copyright Wiley 2019


decellularized extracellular matrix are significant components in the fabrication of cardiac patches (76). Two types of techniques are available to get exact 3D structures: scaffold-based and scaffold-free. A scaffold with ECM material helps to support the tissue regeneration process and improve its reconstitution into functional tissue (77). Decellularized tissue is also used as scaffold material because its structure is like natural tissues (78). The biocompatibility of the scaffolding materials is a key factor in eliminating the risk of transplant rejection. So, the materials or degradation products should be selected carefully (79).

Recently, fully personalized cardiac patches were made by a tissue regeneration technique. The cellular and extracellular matrix were isolated from a biopsy of fatty tissue collected from patients in this investigation. The extracellular matrix was hydrogenized while the cells were being grown to become pluripotent stem cells. Cell-containing bioink formulations were introduced to the 3D printer from the combination of cells and hydrogel, and the cells were efficiently changed into cardiac cells to construct patient-specific immunocompatible cardiac patches layer by layer (80). Nonetheless, these cardiac patches lacked blood vessel networks that matched the patient's vasculature's anatomical architecture. The survival and function of patches after transplantation were dependent on the pre-engineered vasculature within the parenchymal tissue (42, 81–87). The new 3D-printed heart contains cells, blood vessels, chambers, and other structures that are needed for the heart to function normally.

In the study on the development and application of 3D printing techniques using hydrogel as bioink in heart tissue regeneration, the hydrogel, when mixed with the patient's self-cells, can be utilized to print multi-layered, vascularized, and perfusable cardiac patches that perfectly match the patients' immunological, biochemical, and anatomical characteristics. Furthermore, research has shown that the

Fig. 10 Schematic concept illustration of 3D bioprinted hollow tissue fabrication methods: **A** urethra graft, **B** esophagus graft, **C** intestinal epithelium construct, **D** dual-headed extrusion bioprinting, and **E** organoid printing. Reprinted with permission from Galliger *et al.* (48)
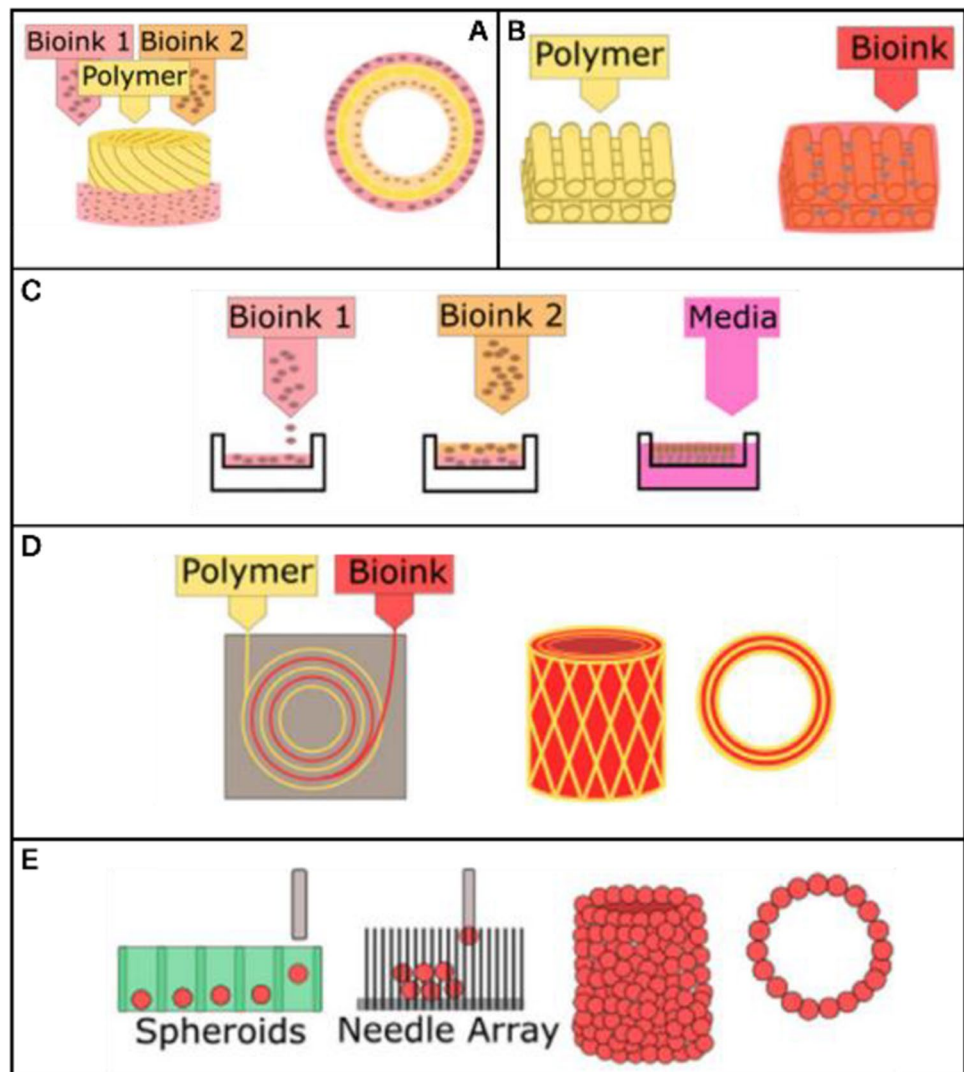
customized hydrogel can be used to print three-dimensional, detachable cellular structures that are linked to complete hearts and their blood veins.

Spider silk One of the inventive approaches to cardiac tissue regeneration is using spider silk, which helps to grow new cardiac muscle tissue. Spider silk produces hydrogels. From this high-grade material, tissue-like structures can be produced via the 3D printing method. Living cells are integrated into these hydrogels, which can provide functional stability to the heart cells. Researchers are mainly interested in the proteins present in the spider silk that give structural and mechanical strength. A study team led by Professor Thomas Scheibel at the University of Bayreuth successfully produced a "bioink" or hydrogel through the mixing of spider silk with mouse fibroblast cells using 3D printing. The gels change rapidly from a fluid to a solid state when flowing through the printer head onto an extrusion surface. This knowledge has been used to effectively produce cardiac muscle tissue using

spider silk scaffolds and cardiomyocytes. The results showed that the bioengineered spider silk is an effective basis for the restoration of heart muscle tissue (89).

Stomach, Intestine, and Bile Ducts

The esophageal is the first site in the gastrointestinal tract (GIT) to demonstrate peristalsis movement, connecting the throat to the stomach. In an adult human, it is a soft tube that's around 25 cm long and has a wall thickness of 3 to 5 mm, depending on whether the esophageal smooth muscle is constricted (90).

Although several tissue-engineered esophageal treatments have been tested, only a handful have used 3D bioprinting technology. Those who have employed 3D printers indirectly did so by making unicellular scaffolds out of PCL and implanting them for vascularization and cellularization. Park *et al.* (2016) first employed a melt extrusion 3D

printer to build a grid structure of PCL (illustration shown in Fig. 10B) (91).

The stomach and intestines both have very similar radial patterns to that of the esophagus, covered by nerve tissue with an inner submucosa and mucosa membrane. The stomach has an extra layer of muscle compared to the other parts of the GIT, as the innermost oblique layer of smooth muscle is present in the muscular externa. The most noticeable difference is in the epithelium. Where intestinal epithelium-specific to absorbing secreted waste and nutrients, the gastric epithelium is mainly designed to protect against acid damage and secrete acid. These epithelia present a significant challenge and role for the tissue engineering field. The stomach has not yet been previously constructed using 3D bioprinting procedures. However, a combined research team from Organovo and Merck created a flattened replica of intestinal epithelium lying on muscle using a patented 3D bioprinting technique (92).

The main objective of this research is to invent better tissue models for toxicology and drug studies and to regenerate a flat structure of the intestines by exhibiting the ability to print cell-laden gels. The human biopsy-derived intestinal epithelial cells (IEC) and human intestinal myofibroblasts (IMF) were added separately in a proprietary bio-ink. Here, the transwell membrane was printed as a layer by the fibroblast ink, and then the epithelial ink was printed as a layer on top (illustration shown in Fig. 10C). Then, this bilayer was matured in culture for 10 days and then examined and tested against different types of drug compounds. Tested tissue showed various epithelial subpopulations like secretory goblet cells and enterochromaffin cells, both of which are very important to mucosal function (92). Immunolabeling indicated that the epithelium had the proper polarization and structure of the tight cellular junctions between the cell membrane transporter and epithelial cells. The approach and analysis employed in this work were constrained due to their exclusive character. This work showed the potential of 3D printing with patient-derived cells, and it also replicated the flat structure of the intestine and epithelialization independent of inward growth from anastomosis, which would allow for the creation of longer intestinal segments. Any mechanical characteristics of the bilayer are not researched in this work, though, and consideration of peristaltic function and muscular structure must be considered in the next steps toward bioprinting an intestinal graft. Other hollow GIT structures, such as the gallbladder and bile ducts, have similar general structures: a simple columnar epithelium-lined central lumen that is bordered by smooth muscle layers. Yan *et al.* (2018) demonstrated that bioprinted cholangiocytes may self-organize into branching tubular structures using a bioink made of cholangiocytes, self-assembled nanofibers, and gelatin (93).

Excretory Organs

The human urinary system relies on hollow and tubular structures to operate effectively, from the Bowman's capsule through the tubules of the renal nephron down to the urethra. The kidney is made up of many tiny tubules, so it is considered and classified as a solid organ. Here, we will discuss only the hollow excretory organs: the urethra, ureter, and urinary bladder.

The ureter is a hollow tube that links the kidney to the urinary bladder in the pelvis region and delivers urine passively through it. The ureter has an epithelium layer that is surrounded by a smooth muscle layer, making it capable of using peristaltic movement for urine transportation (94). The urinary tract shows a different kind of epithelium cell lining than that of other cells from different regions of the body. This epithelium cell is also known as urothelium or transitional epithelium. This urothelium acts as stratified cuboidal epithelium in the relaxed phase and as stratified squamous epithelium in the stressed phase (95). Urothelium cells line the ureters, urinary bladder, and some parts of the urethra (96).

The urinary system is one of the first and foremost effective instances of generating replacement organs, and there are several potential successful examples of tissue engineering techniques (97). Although the 3D bioprinting technique has not been applied in the urinary tract, Zhang *et al.* (2017) printed a multi-layered urethra using an extrusion 3D bioprinting system developed by the Atala Group at Wake Forest University (98) (Fig. 10A). Pi *et al.* (2018) used the coaxial extrusion technique as a much more advanced approach to creating hollow tubular urothelial tissue (Fig. 11) (99).

Later in the same year, Imamura *et al.* used the same previous needle-based technique to 3D bioprint urinary bladder tissue (100).

CLINICAL APPLICATIONS AND THEIR EFFECTS ON QUALITY OF LIFE (QoL)

The medical demands of aging populations, rising unmet demand for organ donors, tendencies toward non-animal testing on treatments employing 3D cell culture platforms, clinical needs in wound care, and joint repair and replacement procedures are all driving developments in the field of bioprinting. The various clinical applications are as follows:

- simulation of tissue for drug development and drug discovery,
- drug toxicity testing,
- tissue engineering for regenerative medicine and prosthetic medical devices, and
- organ transplantation

Fig. 11 A–D 3D bioprinting urethra printing, crosslinking, and immersion in media. Reprinted with permission from Zhang *et al.* (98)
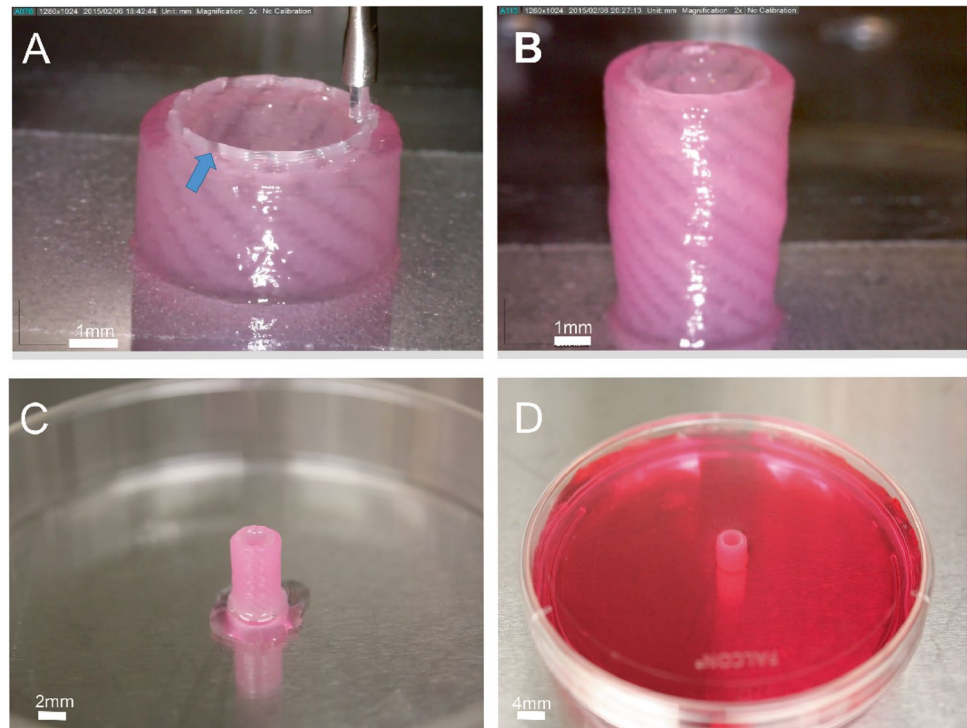
Many elements of health care, including diagnostics (using medical imaging to construct models that help in visualization), surgical planning, and customized medicine, have been proposed to benefit from 3D printing. Bioprinting applications may disrupt current organ and tissue donation arrangements, albeit these applications are likely to be further down the road than other 3D printing uses. 3D printing is now being used or investigated in a variety of therapeutic contexts. As a result, 3D printing has the potential to influence a wide range of health problems. This will undoubtedly increase the QoL of the people.

REGULATION OF 3D-PRINTED HOLLOW ORGANS

Policymakers around the globe want to see more regulation for 3D-printed organs. However, existing regulatory frameworks do not properly match 3D bioprinting. Policymakers must weigh a variety of concerns when deciding how to regulate 3D organ printing. Because the technology is still in its early stages, there is a great deal of ambiguity concerning the actual hazards and ethical problems. One ethical worry is that 3D-printed organs may only be available to the wealthy, while the less fortunate will be denied access. Another issue to consider is security. It is difficult to analyze the safety concerns of 3D printing since it may involve stem-cell technologies and the use of patient's cells for replication. Because stem-cell treatment cannot be tested on a large number of healthy people, clinical trials are limited.

The Food and Drug Administration (FDA) regulates 3D-printed organs in the USA. So far, only general guidelines for 3D printing organs have been issued by the FDA, and these guidelines do not include newer, more complicated bioprinting processes (101).

Health Canada (HC) provided preliminary recommendations to help medical device makers design rules for bioprinting in Canada. According to them, manufacturers seeking bioprinting licenses should be required to submit information about the use of additives in materials, the verification of software for bioprinting design, the method of sterilizing the machines, and the process of safely removing and reusing bioprinting materials and residues.

The European Medical Devices Directive, the Active Implantable Medical Devices Directive, and the In-vitro Diagnostic Medical Devices Directive of the European Union (EU) all govern 3D printing health technology in Europe. Bioprinting devices are classified into numerous risk classifications under the Medical Devices Directive. Devices rated as greater risk are submitted for third-party evaluation and more "stringent" clinical data criteria across the different classes. Implantable devices, such as 3D organs, fall into the highest risk category and require an "independent design dossier review." A design dossier analyzes risk, clinical data, and the technology's compliance with rules and standards.

FINAL THOUGHTS AND PROSPECTS

Our review mainly concentrated on 3D bioprinting, decellularization, and then recellularization, which can minimize the shortage of organs and can effectively overcome many difficulties in the medical field. This method contributes to the development of artificial organs for patients suffering from organ failure. This technique can be efficiently materialized for customized and regenerative medicine for the benefit of human health and social welfare. The working mechanism of 3D bioprinting has many similarities with 2D printing, except for the use of biomaterial, stem cells, hydrogels, and patient-derived cells to create a personalized organ or tissue. The various biomaterial- and bioink-based bioprinters such as extrusion, laser-assisted printers, and stereolithography are the most acceptable bioprinters used in the field of 3D printing technology.

3D bioprinting has the potential to revolutionize individualized regenerative disease therapy. The development of 3D bioprinting has overcome several notable obstacles. Researchers are constantly working on the development of bioprinting techniques and methods. In recent times, scientists have used live cells of the lungs and heart. There is research where the 3D-printed graft is already placed in the animal body, with trials are ongoing. A day is yet to come when patients will give their stem cells and happily wait in their homes to get their personalized organs 3D printed.

The bioprinting community and the pharmaceutical industry have shown a keen interest in 3D-printed organs. Many start-ups dedicated to this field have thrived, and American pharmaceutical behemoths like Johnson & Johnson have taken note. The 3D bioprinting market alone has now grown to a hundred-million-dollar market, with an annual forty-four percent market growth, estimating the market to cross billions in the next five years.

In contrast to a synthetic matrix, which is more likely to be rejected after transplant, 3D bioprinting using hydrogel through tissue regeneration technique can be personalized for the body without evoking much immune response. A few limitations to the applicability of the techniques are the expenditure of the process, stability, and natural shape of the organ. In addition, another huge challenge would be the ethical approval of the government of the country for human organ culture and proper utilization. Again, 3D bioprinting approaches will have the capability to augment the stability and shape of the creation of tissue or organs, and this will be a great opportunity in the pharmaceutical and biomedical fields.

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Declarations

Conflict of Interest The authors declare no competing interests.

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