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3D-bioprinted cardiac tissues and their potential for disease modeling

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Abstract

Heart diseases cause over 17.9 million total deaths globally, making them the leading source of mortality. The aim of this review is to describe the characteristic mechanical, chemical and cellular properties of human cardiac tissue and how these properties can be mimicked in 3D bioprinted tissues. Furthermore, the authors review how current healthy cardiac models are being 3D bioprinted using extrusion-, laser- and inkjet-based printers. The review then discusses the pathologies of cardiac diseases and how bioprinting could be used to fabricate models to study these diseases and potentially find new drug targets for such diseases. Finally, the challenges and future directions of cardiac disease modeling using 3D bioprinting techniques are explored.

Graphical abstract:

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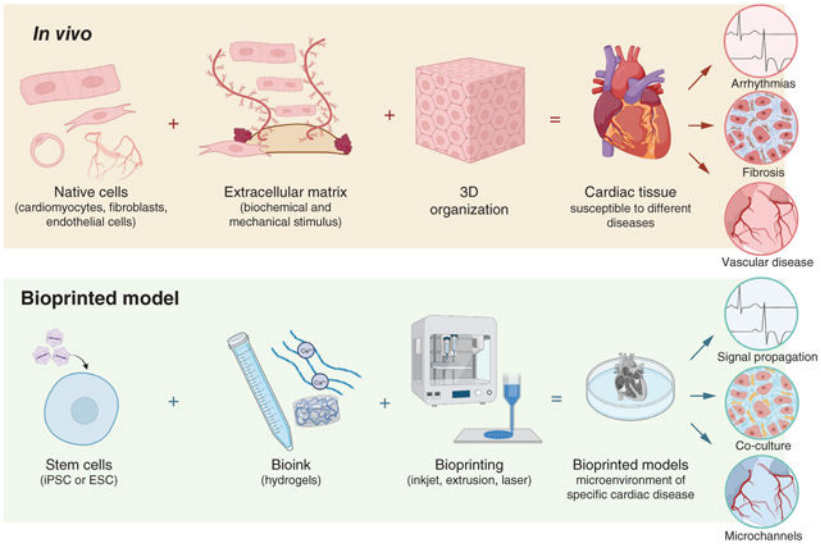

Tweetable abstract:

In this article, the authors discuss how #3dbioprinting can be used to model healthy and diseased cardiac tissue.

Executive summary

- Heart diseases, specifically cardiovascular disease, are the leading cause of global mortality.
- The most common models used to study cardiac diseases include rodent models and 2D cell culture.
- Biomaterials that can be used to mimic cardiac tissue include alginate, fibrinogen, collagen, gelatin-methacrylate, gelatin and other natural and synthetic polymers.
- Healthy cardiac models are being bioprinted using extrusion-, laser- and inkjet-based techniques.
- Cardiac diseases can be grouped into four groups: coronary and vascular diseases, arrhythmias, structural heart diseases and acute conditions such as myocardial infarction and stroke.
- 3D modeling techniques include 3D printing/bioprinting and organoid models.
- Current bioprinted models are mainly being used for regenerative medicine or as a preprocedural tool.
- Developing tissues with complex vascular networks will be challenging, and the biomaterials must not interfere with cardiac action potentials.
- Large quantities of cells are required to print physiologically relevant models, and a variety of cells must be used, which poses its own challenges.

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- 3D models will potentially serve as a tool for personalized modeling; however, this comes with challenges such as standardization, the safety and efficacy of the tissue and cost.
- Bioreactors and machine learning could serve as tools to enhance the reproducibility and scalability of tissues.

Keywords

bioprinting; cardiac tissues; cardiomyocytes; disease modelling; review; stem cells

WHO has classified cardiovascular disease as the leading cause of global mortality [1]. It was estimated that in 2019, 17.9 million people died from heart disease, representing 32% of all deaths globally [1]. In Canada alone, about 14 adults die every hour due to a diagnosed heart disease, making it the second leading cause of death in Canada [2], whereas in the USA, heart disease is the leading cause of death for both men and women [3]. It is also estimated that heart disease costs the USA $220 billion (USD) each year, which includes the cost of healthcare services and medication [3]. Heart disease can be classified by the function or structure of the heart it affects, including the arteries, blood vessels, ventricles, atria and muscles. First, coronary artery and vascular disease are the most common type of cardiovascular disease, and they occur when the coronary arteries and other blood vessels are blocked or narrowed, respectively [1]. Second, heart rhythm disorders (arrhythmias) cause the heart to beat too fast, too slow or irregularly and this causes sudden disruptions in blood flow [1]. Third, structural heart diseases are characterized as structural defects in different parts of the heart, including the valves, walls and muscles [1]. These diseases include cardiomyopathy, congenital heart disease and heart valve disease. Finally, heart conditions such as heart attack, stroke and heart failure are also a leading cause of death, but they are typically acute events that are caused by other heart conditions [1].

There are a variety of cardiac models that have been used to study new drugs and the pathophysiology of cardiac diseases. *In vivo* studies using animals such as mice, rats, rabbits, canines, sheep and pigs are the most widely used [4]. Out of all these animals, rodents (mice and rats) are the most popular because they are easier to handle, they are lower in cost and, since they have a short gestation period (~21 days), genetically modified models can be created in a short period of time [4]. Although studies using rodents provide valuable information about human cardiac diseases, some cardiovascular parameters are starkly different from humans [4,5]. For example, a rodent's heart rate is between 310 and 840 b.p.m., whereas humans have an average heart rate of 72 b.p.m. [4]. A rodent's heart also has different excitation and contraction properties when compared with a human heart, and the body weight of rodents (0.02–0.063 kg) is also very different from the average human weight (50–86 kg) [4]. These are all significant differences that can limit the translation of findings from rodent studies to humans. Larger animals such as canines, pigs and sheep would more closely resemble the human heart; however, the cost of acquiring these animals is significantly higher, making them less desirable [4]. The zebrafish blastema model has emerged as a useful cardiac model to study heart regeneration and human cardiovascular diseases [6,7]. Zebrafish are transparent and can easily be genetically manipulated, which makes them easy to work with for

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phenotypic assays [6]. However, the zebrafish heart has only two chambers with a single atrium and ventricle, so it does not directly mimic the human heart [6]. Researchers and pharmaceutical companies have also utilized 2D monolayer *in vitro* models to study cardiac diseases and drug efficacy in preclinical trials [5]. However, these models lack the complex microenvironment, physiological characteristics and functions of cardiac tissue. Thus, 3D-tissue models, including organs-on-chips, 3D-scaffolds and 3D-bioprinted models have grown in popularity, since they address the limitations of 2D models [5,8–11].

3D bioprinting has become one of the most advanced techniques to mimic the microenvironment of cardiac tissue [12]. This method can generate physiologically relevant models that can be used as an *in vitro* system to evaluate biological responses. This multidisciplinary technique is low-cost and efficient, and it allows researchers to generate highly defined geometries using biomaterials while maintaining cellular viability and functionality [13]. In general, the process of bioprinting consists of the simultaneous deposition of cells and biomaterials in a layer-by-layer fashion, forming a construct that can morphologically and structurally mimic native tissue architectures [14]. A variety of techniques are being used for cardiac tissue engineering, which include inkjet, extrusion and laser-based bioprinting [15,16].

One of the obstacles in bioprinting is finding a balance between printability and biocompatibility. The mechanical properties of the biomaterials must be compatible with the printing technique to achieve the desired resolution and it must mimic the native tissue to allow for the required dynamic cell behaviors [17]. For this reason, the fabrication of tissues requires suitable bioinks, a solution developed specifically to support cells and allow for proper printability. The distinct and complex biochemical composition of each tissue requires unique components to provide the necessary cues to maintain cell phenotype, viability, function and maturation [18]. However, for cardiac tissue, studies suggest that bioinks must also be electrically conductive to generate a functional model [19]. Most bioinks used in cardiac tissue engineering are naturally derived from humans or other animals. However, there are synthetic bioinks that some researchers have used to create cardiac models. For example, polyvinyl alcohol has been used as a sacrificial bioink, and a polyester urethane urea cardiac patch, with stem cells, was developed and implanted in a mouse model [20,21]. This review focuses on the most commonly used bioink components, including collagen and fibrin, because these are components that are naturally found in a human heart.

Current reviews focus on the challenges faced when bioprinting cardiac tissues in general, but there is a lack of literature that describes the potential challenges when bioprinting cardiac disease models. Thus, this review aims to discuss the current literature on how cardiac tissues have been bioprinted and the specific challenges faced when trying to 3D bioprint models of cardiac diseases, including arrhythmias, vascular disease and structural disease. First, the authors describe the important mechanical, chemical and cellular properties of cardiac tissue and how these properties can be mimicked in 3D bioprinted tissues. Next, they review how current healthy cardiac models are being 3D bioprinted. They also introduce the pathologies of cardiac diseases and how bioprinting could be used to create models to study these diseases and their potential challenges. Finally, future directions

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of cardiac disease modeling using 3D bioprinting techniques are discussed. This review aims to provide a concise perspective on bioprinting cardiac disease models, with the hope that it will help others understand potential challenges, so that better solutions can be developed. If patient-specific cardiac disease models can be engineered, it will reduce the need for animal models, which do not directly mimic the human heart, and thus will potentially increase the success of future therapies.

Properties of cardiac tissue & their translation to bioprinting applications

Figure 1 shows the different components of the heart and how bioprinting can be used to replicate these characteristics. This section discusses the properties of cardiac tissues in depth.

Cells found in cardiac tissue

To pump blood, individual cardiomyocytes synchronously contract and relax to generate rhythmic contraction–relaxation cycles. Atrial and ventricular cardiomyocytes form the muscular walls of the cardiac atrial and ventricular chambers, respectively. For blood to flow through the chambers, these cells exhibit different action potential (AP) properties [22]. Pacemaker cells, also known as nodal cardiomyocytes, are responsible for generating and dictating the heart's rhythm, while Purkinje fibers are responsible for the orientation of the electrical stimulus throughout the heart [22]. Cardiac fibroblasts are one of the most abundant cells in the myocardium [23]. These cells surround cardiomyocytes and bridge tissue layers, contributing to the biochemical, mechanical and electrical properties of the heart [24]. Due to the proliferative potential of fibroblasts and their ability to synthesize extracellular matrix (ECM) proteins, the density of these cells in cardiac tissue must be kept at equilibrium; otherwise, a fibrous environment can emerge [24]. Finally, endothelial cells also play an important role in heart function. Forming the inner layer of blood and lymphatic vessels, endothelial cells can control vasomotor tone, blood flow, vascular permeability, leukocyte trafficking and angiogenesis [25]. Due to these functions, these cells are in constant communication with cardiomyocytes and fibroblasts, promoting angiogenic signaling, inflammation and ECM deposition [26].

Both 2D and 3D models utilize primary cells, cell lines or stem cells [5]. Primary cells are directly obtained from human tissue or that of other animal species and are not genetically or virally transformed, which allows them to maintain the cellular behaviors found *in vivo* [5]. However, primary cells have a short life span and limited proliferation capacity, and they require invasive surgical techniques [5]. For these reasons, cell lines are the most utilized cells in *in vitro* models, since they have unlimited proliferation capacity and are standardized [5]. However, their cellular behavior can easily change depending on the passage number and culture conditions [5]. Due to the limitations of these cells, stem cells have grown in popularity for modeling cardiac tissues due to their ability to differentiate to relatively pure (50–90%) cardiomyocytes (CMs) [5]. Their proliferation capacity and maturation can be adjusted depending on a variety of factors [5]. However, stem cells derived from embryos (ESCs) present various ethical issues and are difficult to obtain. Therefore, human-

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induced pluripotent stem cells (HiPSCs) are more commonly used because they are directly generated from somatic cells using the Yamanaka factors [27].

The ECM of cardiac tissue

In cardiac tissue, the ECM has structural functions by providing support and strength for the cells' contractile movement [28]. The ECM also has nonstructural functions by accommodating multiple proteins with growth factors and cell receptor-binding properties [28]. Although the ECM has a wide range of roles in the maintenance of cardiac tissue, its hallmark is the ability to support a reliable behavior during events of dynamic mechanical load, such as pulsatile blood pressure and flow [28]. This relation is known as mechanobiology, and its effects on homeostasis are directly related to the proteins that compose the cardiac ECM [29]. In general, the ECM of cardiac tissue can be viewed as a basement membrane and interstitial matrix [30]. The basement portion of the ECM contains specialized molecules, such as fibrin, collagen type IV, laminin, hyaluronic acid and proteoglycans, which promote cellular functionality through interactions with surface receptors [31]. Fibrillar collagen (types I and III) and elastin make up the interstitial matrix of cardiac tissue, and these components are responsible for the structural and mechanical integrity of the tissue [32].

Cardiac tissue has several mechanical characteristics, all of which play important roles. Stiffness and topography show a significant impact on the behavior of cardiac cells [33]. Stiffness can be defined as a material's resistance to deformation by an applied force, and it can be measured by Young's modulus. Studies in rat myocardium have found that stiffness can significantly increase in diseased environments, such as infarcts and fibrosis [34]. Topography can be defined as the structural characteristics of the ECM at the surface level. Cardiac tissue has specific topography characterized by the cells' parallel alignment, known as a Young's modulus, which provides structural stability and tensile strength [35].

Biomaterials needed to mimic cardiac tissue

In designing bioink, it is vital that there is an understanding of the chemical, physical and mechanical properties, so that cardiac cells can be properly supported. Among the biomaterials used to mimic cardiac tissue and provide proper printability, some options seem to recur throughout studies, such as gelatin methacrylate, alginate, fibronectin and gelatin [12–14,17,21,36–39].

Gelatin methacrylate—Gelatin methacrylate is an engineered, gelatin-based material that has become popular in tissue engineering due to its versatility, biocompatibility and ability to photo cure [40]. When associated with high-resolution techniques, such as laser-based bioprinters, gelatin methacrylate was shown to be able to generate scaffolds with complex microarchitecture and able to reproduce stimulus of native cardiac ECM topography [41].

Alginate—Alginate is a natural polysaccharide derived from the cell walls of algae. Due to its unique properties and renewable origin, this material is becoming one of the most popular components of bioinks [42]. However, cells do not adhere well to this material; therefore, it has limited capacity for the maintenance of cardiac cells [43]. In addition, the use of

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alginate in cardiac implants suggests that the material is poorly conductive and can impede the propagation of cardiac AP [44]. These drawbacks can be solved when this material is associated in a bioink with other components. It was demonstrated that extruded constructs of alginate and platelet-rich plasma exhibit suitable mechanical properties and the viability of cardiac cocultures [21]. Also, in laser-based bioprinter applications, alginate has shown promising results when associated with carbon nanotubes, providing proper electrical and mechanical properties [19].

Fibrin—Fibrin is a fibrillar protein of extreme importance in the blood clotting cascade. Fibrin is a great biomaterial because it replicates the ECM and stimulates cell adhesion, proliferation and differentiation [45]. Moreover, fibrin is physiologically biodegradable through a mechanism that allows ECM replacement and integration when new tissue is formed [46]. This fibrillar protein exhibits structural integrity, having high tensile strength and adhesion strength, enabling cells to adhere to it, along with its biodegradability in soft tissues [46]. In 3D-bioprinted cardiac tissue, fibrin has been shown to support and help orientate iPSC-derived CMs [13]. Using a droplet-based bioprinter, fibrin has also been shown to help CMs proliferate and beat synchronously [14].

Gelatin—Gelatin is a natural material derived from collagen that is known for its large number of applications due its ability to solubilize in warm water and form physical hydrogels at low temperatures. For bioprinting, especially extrusion-based, this feature has been useful because bioink viscosity can be changed with temperature [47]. During the printing process, the gelatin physically gelatinizes and temporarily stabilizes the hydro gel scaffolds, reducing shear stress, and during incubation, this material melts [48]. Also, gelatin has enzymatic cleavage sites, which cells can degrade, so this material can be used as a temporary scaffold until the cells secrete their own ECMs [48]. However, due to the low mechanical stability of gelatin, it is usually modified with other components to be compatible with tissue-engineering applications [49]. In cardiac bioprinting, combinations of gelatin with fibrin and fibrinogen have shown promising results [36,38]. Additionally, gelatin can act as a sacrificial material, which makes it useful when printing complex architectures [37].

Collagen—Collagen is an abundant protein in the cardiac ECM and is known to promote cellular adhesion and mechanical strength, and it enables structural organization [50]. Although these reasons popularize collagen over cardiac tissue engineering, its use in bioprinting faces difficulties due its poor self-supporting property and low viscosity [19]. Unmodified collagen gelation is typically achieved through self-assembly driven by temperature, which is difficult to control [17]. For this reason, collagen is often modified or associated with other materials. To demonstrate the potential of this material when this drawback is counteracted, a modified pH change to drive collagen self-assembly was used to replicate a functional extruded construct with patient-specific anatomical structures [17]. Also, a newly developed form of type I collagen, named Viscoll, has shown promising viscoelastic properties in extrusion bioprinting, making it a potential alternative for cardiac tissue models [51].

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Other materials—Other natural and synthetic polymers have shown interesting properties for cardiac tissue modeling. The combination of gelatin and hyaluronic acid seems to improve cardiac maturation and longevity in animal engraftments [52], suggesting this long-chain disaccharide could be useful for long-term models. Other interesting materials for cardiac tissue engineering include silk fibroin hydrogels; although these can form nanocrystals, when crosslinked with other materials, this natural polymer improved the mechanical and biological properties of bioprinted constructs [53]. As a result of its hydrophobic composition and polarity, silk fibroin offers a promising alternative to improve the properties of printed models in terms of strength, resistance and longevity.

Thermoplastics have been used, though not widely, for bioprinting cardiac tissue. Synthetic biodegradable polymers such as polylactide, polyglycolic acid and their copolymer, polylactide-glycolic acid, are attractive materials due to their strong mechanical properties, processing flexibility and low immunological responses [54]. Additionally, in one study, polylactide-glycolic acid associated with carbon nanofibers could align CMs while improving electrical and mechanical properties [55]. Also, surface treatments can help improve cell adhesion and proliferation [56]. As a result, these polymeric materials hold great potential for improving specific properties and developing more accurate models of cardiac tissue.

How healthy cardiac tissues are 3D bioprinted

This section discusses the methods for 3D bioprinting cardiac tissues. Figure 2 provides a visual comparison of these techniques for easy reference.

Inkjet-based bioprinting

The origins of 3D bioprinting can be traced back to the initial stage of cellular bioprinted structures using inkjet printing technology, which is an approach first introduced by Thomas Boland *et al.* [57–59]. Inkjet-based bioprinting (IBB) is a method used frequently for biological applications. This technique deposits a defined volume of a cell-encompassing bioink onto a supporting material through distinctive energy sources (thermal and piezoelectric) and a droplet-based mechanism [16,58,60]. Effective deposition is contingent on the bioink's possession of certain physical properties, including viscosity, density and surface tension [61]. The necessity for certain bioink properties produces limitations, as encountered by the bioink's low-viscosity requisite, leading to constructs with deficient mechanical properties [15,57]. Even so, IBB can generate high-resolution 3D structures swiftly and at a low cost, as well as exhibit cellular viabilities of 80% [15,58,60,61].

The use of IBB in the fabrication of functional cardiac tissue was demonstrated by Xu *et al.* [62]. In this study, the authors printed layered 3D cardiac constructs with a particular "half heart" design composed of alginate/gelatin gels encapsulating CMs and crosslinked with $CaCl_2$. Having undergone electrical simulations, the 3D cardiac pseudostructures exhibited functional excitation–contraction pairing, with visible rhythmic contraction of CMs within the 3D-printed structures, along with recurrent beating of the structures as a whole [62]. These results demonstrate IBB's capability in producing functional cardiac constructs.

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Extrusion-based bioprinting

Extrusion-based bioprinting (EBB) is the most popular 3D bioprinting technique when generating cardiac structures [15,63,64]. EBB utilizes a computer-controlled system to eject bioink strands through a nozzle and onto a surface, creating a layer-by-layer 3D structure [16,65]. Typically, the biomaterial is inserted into a metallic or plastic syringe and extruded by route of a pneumatic, piston-driven or screw-driven force [66]. The pneumatic mechanism utilizes air pressure to achieve extrusion, as opposed to the mechanical technique (i.e., piston- or screw-driven), which uses vertical and rotational forces [61]. EBB is characterized by its efficiency in depositing biomaterials with high cell densities (10^8–10^9 cells/ml), similar to physiological cell densities [15]. Using EBB's multinozzle features and rapid print speed, intricate structures can be created using a variety of biomaterials and cell types [16]. There are, however, certain limitations of EBB, including low resolution (200 μm; contrasting laser- or inkjet- based processes), low cellular viability due to shear stress and highly viscous bioinks, causing harm to cellular function/morphology [15,66].

In a study conducted by Wang *et al.*, the authors implemented EBB in the fabrication of functional cardiac structures using a three-axis stage system with multiple extruding modules containing pneumatic pressure control [65]. Cardiomyocytes isolated from infant rat hearts were encapsulated in a fibrin-based bioink, composing the hydro gel. The hydro gel, along with a sacrificial hydro gel and a sustaining polymeric frame, were extruded, producing cardiac structures exhibiting coordinated contraction while in culture, suggesting that the cells were mature. In a different study, Zhang *et al.* created vascularized cardiac tissue through EBB [67]. Endothelial cells (embedded in microfibrous hydro gel scaffolds via bioprinting) were granted the ability to migrate by using a composite bioink, generating a confluent endothelium layer, with the assembly of the endothelial cells echoing the architecture of blood vessels [67].

Laser-based bioprinting

According to Agarwal *et al.*, laser-assisted bioprinting (LAB), or laser-induced forward transfer, employs a high-intensity laser, which impels the bioink droplets within a noncontact mode [16]. There are three primary components within LAB, including a pulsed laser beam; a target plate (the ribbon), which is covered by the bioink; and a receiving substrate. By means of a transparent ribbon, the laser beam passes through it and reaches the substrate, expelling a cell-loaded bioink onto the substrate. The substrate is typically covered with hydrogels, minimizing the impact of preceding situated droplets. Programmable features of LAB include laser frequency, intensity and motion control. With LAB being a nozzle-free process, nozzle clogging is avoided. LAB allows for high cell densities (~10^8 cells/ml) owning high resolution (10–100 μm) and permits an ample range in biomaterial viscosities (1–2000 mPa/s) [16]. The LAB technique, however, is also accompanied by certain limitations, including its limited capability of expelling various cell types. LAB can also be expensive, is a sluggish process, and is commonly characterized by small structures with limited clinical applications [16].

In a study conducted by Gaebel *et al.*, the authors created a cardiac patch and seeded human umbilical vein endothelial cells and human mesenchymal stem cells on a polyester

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urethane urea cardiac patch [20]. Cultivation of the cardiac patches was performed *in vitro* or transplanted *in vivo* onto the portion of the heart being infarcted. The results conveyed modified growth characteristics of cocultured human umbilical vein endothelial cells and human mesenchymal stem cells, making it possible to achieve an increased vessel formation. Prominent functional improvement of infarcted hearts after transplantation of a laser-induced forward transfer tissue-engineered cardiac patch was also noted [20].

Cardiac diseases & modeling techniques

Coronary artery & vascular diseases

Coronary artery disease (CAD) is caused by the blockage or narrowing of coronary arteries, which is commonly due to the buildup of plaques (atherosclerosis) [1]. This causes a mismatch between myocardial oxygen supply and demand. Other common vascular diseases include cerebrovascular disease and peripheral arterial disease, which affect the blood vessels supplying the brain and arms/legs, respectively [1]. The treatment for these diseases includes revascularization and various pharmacological therapies using antiplatelet agents and statins [68]. Revascularization is where blood flow is restored to the heart by performing an angioplasty or stenting procedure [69]. 3D bioprinting has gained popularity in revascularization procedures, specifically with stenting. For example, Lu *et al.* 3D printed a bioresorbable stent with the goal of treating cerebrovascular disorders [11]. They developed a novel stent that enabled antistenosis and disappeared after vessel endothelization [11]. Endothelial cells were also seeded in the stents and good proliferation capabilities were observed [11]. 3D bioprinting has been used for regenerative purposes with the idea of creating patient-specific tissue or stents that can be implanted. However, there are no 3D-bioprinted models to study diseases or new drug targets. These studies are usually performed in animal models such as rodents or porcine. 3D printing, on the other hand (no cells or biomaterials), has been used extensively to create models of complex coronary anomalies, which have been used to simulate potential vascular surgical procedures [70–72]. Aside from 3D-printed models, organoid models have also been used as a potential tool for studying cardiovascular diseases. For example, a study by Liang *et al.* developed vascularized cardiac organoids by differentiating hiPSCs via the Wnt signaling pathway to cardiomyocytes and endothelial-like cells [73]. Their chambered model exhibited more mature membrane potentials and it proved to be a better model for studying cardiotoxicity [73].

Arrhythmias

Cardiac arrhythmias can be classified into two groups, bradyarrhythmia and tachyarrhythmia, both of which are caused by abnormalities in electrical impulses of the myocardium. Bradyarrhythmias are slow heart rate arrhythmias (<60 beats/min) and there are two main types: sinus bradycardia, which originates from the sinus node, and atrioventricular blocks, which are characterized by an interruption or delay of the electrical signals between the atria and ventricles [5]. On the contrary, tachyarrhythmias are fast heart rate arrhythmias (>100 beats/min), which can originate in the sinoatrial node, atrial myocardium or atrioventricular node or below the atrioventricular node [74]. Common subtypes of this disease include atrial fibrillation, atrial tachycardia, ventricular tachycardia

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and atrial flutters [74]. Rhythmic disorders have mainly been studied in 2D models and animal models [74]. However, a recent study developed human 3D microtissues generated by embedding HiPSC-derived CMs in a microwell system [75]. After disrupting gap junctions using cyclodextrin, the authors found that the microtissues started to exhibit rhythmic disorders [75]. Although this study did not use 3D bioprinting techniques, it has been suggested that 3D bioprinting may be useful when developing antiarrhythmia drugs because models can be engineered using cells derived from patients with a genetic predisposition to arrhythmias [76].

Structural heart diseases

Heart diseases can vary and may present themselves due to other health conditions or they may be present at birth. The latter are known as congenital heart disease, and it affects about one in four children [77]. There are varying levels of severity from mild to critical; for example, atrial septal defect can be considered a mild congenital heart disease because it is caused by a small hole in the wall dividing the right and left atria [78]. On the other hand, coarctation of the aorta is considered a critical congenital heart disease because a portion of the aorta is abnormally narrow, which prevents oxygen-rich blood from being sent to the rest of the body [78]. Cardiomyopathies, another type of structural heart disease, affects the heart muscle by causing either the walls of the heart to thicken (hypertrophic) or the chambers of the heart to become too large (dilated) [79]. These diseases can be acquired from other pre-existing conditions such as arrhythmias, long-term blood pressure or other health issues, but they can also be inherited [79]. There are several components to managing cardiomyopathies, including pharmacological approaches and lifestyle changes. However, severe cases require heart transplantation, ablation procedures or surgically implanted devices. 3D bioprinting could serve as an alternative approach for some of these severe cases. For example, Park *et al.* developed a stem cell-laden 3D-bioprinted cardiac patch, which was used for ischemic cardiomyopathy caused by myocardial infarction (heart attack) [80]. After a heart attack was induced in a mouse model, the patch reduced scar tissue formation and improved cardiac function [80]. Another study developed a 3D cardiac coculture model containing CMs, fibroblasts and microvalvular endothelial cells to study the affects of microgravity [81]. The authors also suggest that the model can be used to study cellular crosstalk in cardiac atrophy to better understand the disease pathology [81]. 3D heart organoid models have also been developed by Lewis-Israeli *et al.* to study congenital heart defects [82]. This group differentiated hiPSCs using a three-step Wnt signaling modulation procedure to develop a heart model. Their multicellular models developed chambers and complex vasculature, which were used to re-create a metabolic disorder that is associated with congenital heart defects [82].

Potential challenges when 3D bioprinting models of cardiac diseases

Figure 3 shows how different types of heart disease can be modeled using bioprinting. This section details the challenges of modeling these different types of diseases.

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CAD & vascular disease

Vascularization is the most important factor when trying to mimic vascular-based diseases such as CAD. The most common strategy when developing vascularized tissue is using iPSCs from patients and differentiating them to CMs and endothelial cells to generate a coculture model [13,37,38]. For the bioink, hydrogels containing a patient's ECM have been shown to generate parenchymal cardiac tissue and blood vessels [37,38]. Natural bioinks such as alginate, collagen and fibrinogen have also demonstrated the ability to support multicellular tissues [13,17,21]. However, due to the complexity of human vascular networks, one of the major challenges when engineering vascularized tissue is the poor resolution of the bioprinters. To overcome this, the use of freeform reversible embedding of suspended hydrogels (FRESH) or sacrificial materials has been useful [17,21]. Using the FRESH technique and modified collagen to crosslink through pH, a 20 μm filament resolution was generated and shown to provide rapid cellular infiltration and microvascularization [17]. Also, studies using polyvinyl alcohol as sacrificial material have shown improvement over the flexibility of printed construct, as well as the ability to generate microfluidic channels for endothelial vascularization [21]. Another challenge to overcome is how to arrange the cardiac cells in a way that is physiologically relevant [35]. Hydro gel frames are a new strategy used to orient cells in a desired 3D shape [13,38]. Although a lot of progress has been made in producing vascular tissues, this challenge continues to be one of the main limitations when generating diseased cardiac models, specifically for vascular disease [39].

Arrhythmias

The rhythm of the heart is controlled by cardiac APs, which cause coordinated contractions of CMs, followed by relaxation. These cardiac APs are a result of cell-membrane channels opening and closing, releasing ions and causing AP signals from one cell to another [83]. Some arrhythmias are caused by changes in ionic currents through the Na^{+} or Ca^{2+} ion channels [83]. For this reason, when engineering an arrhythmia model using bioprinting techniques, it is important that the biomaterials and printing technique used do not interfere with ionic currents. As mentioned previously, alginate is a biomaterial that has great printability characteristics, but it can interfere with the propagation of APs and has poor conductive properties [44]. Therefore, in arrhythmic models, it may be best to exclude alginate as a biomaterial and incorporate collagen or gelatin. On the other hand, if alginate is necessary, other electroconductive materials can be added to the bioink, so that signals can be transmitted cell to cell. A fine balance would also need to be found where enough alginate is added, so that the biomaterial is printable, and the concentration of the conductive material is adequate to allow for AP signals to propagate. For example, Roshanbinfar *et al.* incorporated PEDOT:PSS (an electroconductive polymer) into a collagen–alginate hydro gel, making a fibrous microstructure that was similar to native cardiac ECM [84]. It was found that the primary CMs had improved maturation and beating properties [84]. For the cell source, cells derived from patients with a family history of arrhythmias may be the most ideal for disease and drug studies [76]. To extend, hiPSCs could be reprogrammed from the somatic cells of a patient and differentiated to CMs, so that a 3D microphysiological model could be developed, allowing disease progression to be studied and new antiarrhythmic drugs to be found [76].

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Structural heart diseases

To model structural heart diseases such as cardiomyopathies and other neonatal diseases, it is more important that a human-size physiological model be created rather than a microphysiological model. This is because structural irregularities typically occur between different chambers of the heart, so all these components should be mimicked in the model. In this case, FRESH bioprinting may serve as a powerful tool because larger and more complex models can be created [85]. Additionally, FRESH bioprinting has higher resolution, allowing vascular architectures to be constructed [85]. Patient-specific models can also be constructed by using high-quality clinical images of the heart, segmenting it into layers and bioprinting each layer using either laser-based bioprinters or extrusion-based bioprinters [86]. This would allow researchers to study uncommon structural diseases that are patient-specific, so that preprocedural planning, device sizing and disease studies could be conducted [86]. Apart from the bioprinting technique, modeling of these diseases would require a multitude of cell lines, including CMs, fibroblasts, endothelial cells, pacemaker cells and others, so that the full heart structure could be accurately produced. Also, various biomaterials would also need to be incorporated, so that each chamber of the heart and myocardium could be mimicked. This is a large feat that will most likely require multiple biomaterials and bioprinting techniques.

Acute heart conditions (stroke & myocardial infarction)

Although strokes and heart attacks occur due to other, pre-existing heart conditions, the end stage of these acute events result in the damaging of the myocardium, which can cause an increase in arrhythmic disorders and reduce cardiac functionality [87]. During these events, the release of noradrenaline by the adrenergic nervous system increases in an attempt to restore cardiac functionality by augmenting its contractility, which can further damage the tissue [87]. For this reason, a potential strategy to study these acute conditions is to explore the cross-communication between the adrenergic nervous system and damaged myocardium [88]. To date, no bioprinted models have been developed to study myocardial infarction. Instead, 3D studies using spheroids have successfully mimicked the desired environment [89]. To achieve this, a coculture model containing cardiac cells was exposed to a gradient of oxygen concentrations; then noradrenaline was added to the spheroids to induce an apoptotic response and generate “infarction gradients” that were able to mimic zones of infarcted cardiac tissue. With this model, it was possible to observe a reduction of calcium-handling protein expression, CM death and fibrosis, allowing the assessment of responses to clinically relevant drugs [89]. In this way, 3D bioprinting strategies could be incorporated into this approach to include mechanical loading and other conditions that better replicate the functional characteristics of these conditions. Although multiple cell lines can be used when 3D bioprinting, the inclusion of immune cells is still challenging due to the complexities of the *in vivo* pathways, and the effects of reperfusion injury are difficult to replicate (Table 1) [17,21].

Future perspective

In terms of 3D bioprinting cardiac tissues, we speculate that cardiac models will become more intricate with vascular networks and the inclusion of immune cells, which will allow

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researchers to better mimic diseases such as CAD, arrhythmias and acute heart conditions. This will require researchers to merge various bioprinting techniques simultaneously to accurately reproduce the complexities of human cardiac tissue.

Additionally, physicians have been moving toward personalized medicine to better identify patient-specific treatment, and 3D-printed models using patient-derived iPSCs can serve as a tool for these personalized models. Also, autologous hiPSC cardiac models could serve as transplantable tissues with potentially lower immune rejections, since the patients' own cells would be used. As an example, recent clinical trials are transplanting patient-derived MSCs for ear restoration, which paves the way for implanting cardiac tissues. Despite this progress, there are several challenges associated with 3D bioprinting in clinical trials. One of the main challenges is ensuring the safety and efficacy of the printed tissue. Due to the relative youth of 3D printing technology, there is not much standardization in the field, which makes it difficult to ensure that printed tissue meets safety and efficacy requirements. Furthermore, it is difficult to predict potential complications or side effects associated with 3D-printed tissue due to a lack of understanding of its long-term effects. Moreover, the technology is expensive and complex, and the 3D bioprinting process is highly specialized and technical, requiring significant investment in equipment, materials and expertise.

With respect to model development, implementing this technology poses a challenge due to the intrinsic difficulty of growing cells, as well as incubating and maintaining printed constructs, processes that require extensive human involvement and limit the development of human-size models. Consequently, improvements in bioreactor use will be necessary to overcome these limitations and enhance reproducibility and scalability. In addition, to achieve functional cardiac models, bioprinting is an essential process that permits the incorporation of necessary elements in fabricating desired architectures and specific functions. Thus, optimization of the printing parameters is crucial when creating viable tissues. In order to overcome these difficulties, machine learning is a promising approach. Supervised and unsupervised algorithms have proven robust computational capacity, allowing an association among process, material and performance with regard to the bioprinting process, thus demonstrating the possibility of utilizing machine learning in enhancing manufacturability and the quality of attained structures [90]. With the complexity of cardiac tissue, it is vital to optimize the bioprinting process and identify standard printing parameters that will achieve high throughput and consistency of developed models.

Conclusion

3D bioprinting serves as a powerful tool for generating models of cardiac tissues especially when combined with stem cells. This process enables the study of cardiovascular diseases with several advantages for modeling such diseases in comparison with 2D cell culture and animal models. Future work will address challenges with this process, including scaling up cell culture production and translating this work for clinical applications.

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combined with freeform reversible embedding of suspended hydrogels, high-resolution collagen constructs were bioprinted successfully with functional results.

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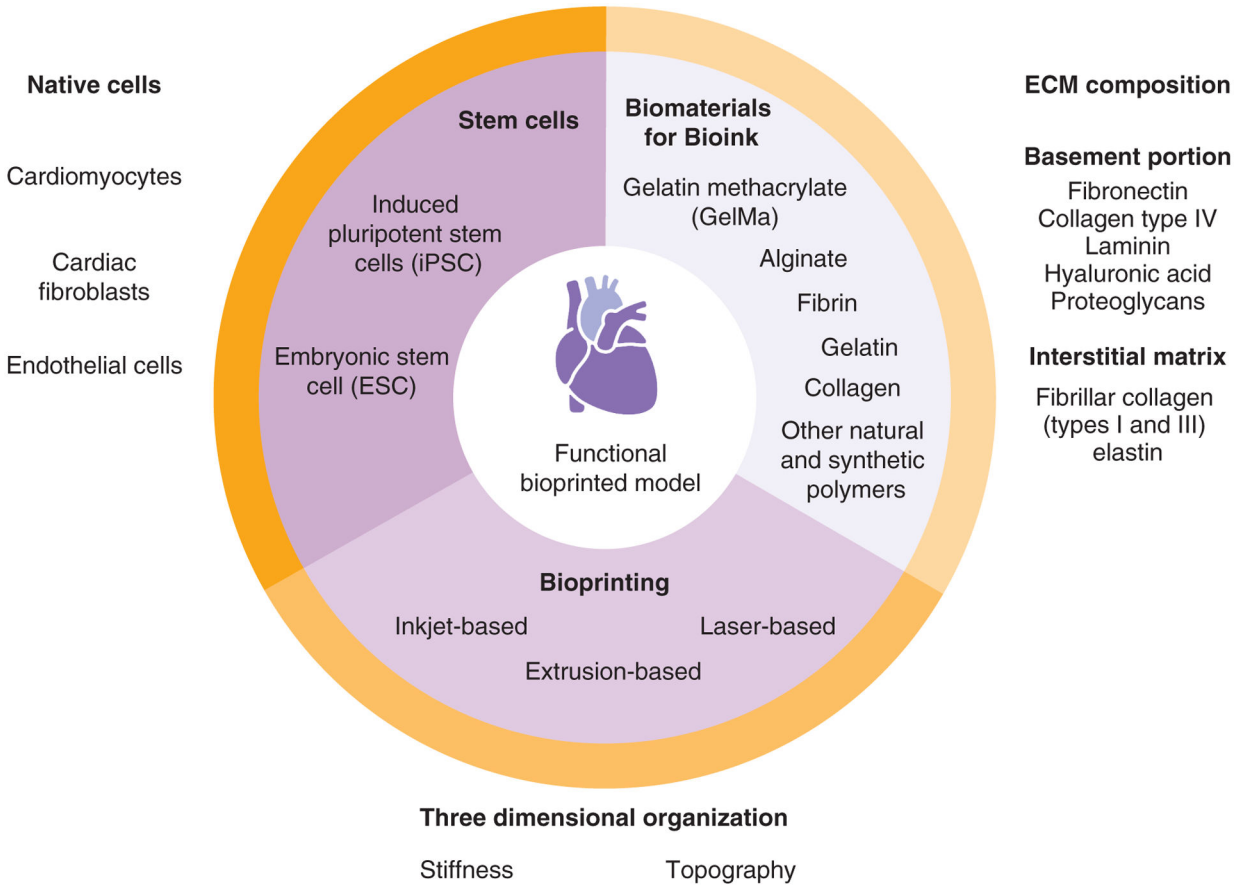

Figure 1. How the native properties of cardiac tissue, including the native cells and embryonic stem cell composition, can be translated to a functional 3D bioprinted model.

ECM: Extracellular matrix.

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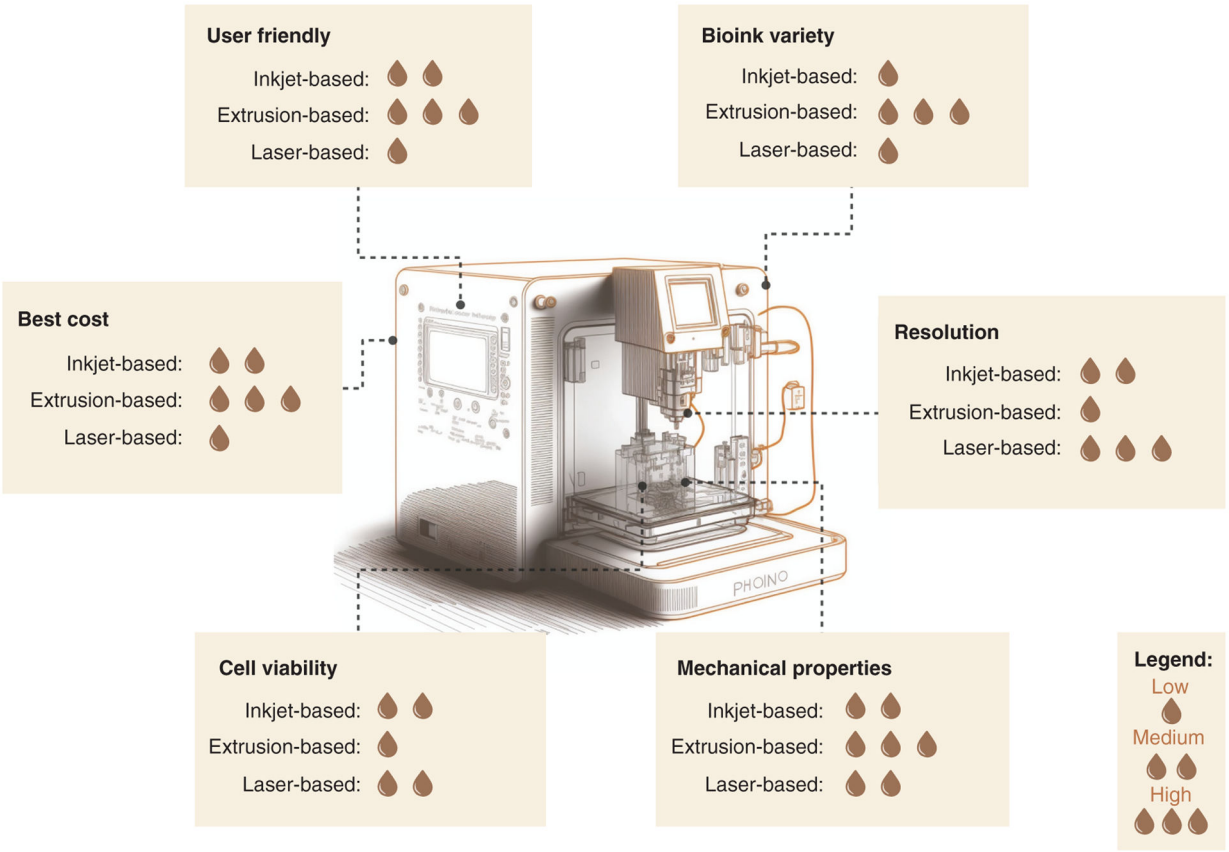

Figure 2. Comparison of inkjet-, extrusion- and laser-based bioprinters for cardiac model development.

The highest cell viability was found in inkjet- and laser-based bioinks, whereas extrusion-based had the lowest. For mechanical properties, the extrusion-based provided more options. For resolution, the best was laser-based, followed by inkjet- and finally extrusion-based. For bioink variety, the best was extrusion-based bioprinters. The most and least user-friendly were extrusion- and laser-based bioprinters, respectively. Finally, the most affordable was extrusion-based, followed by inkjet and laser bioprinters.

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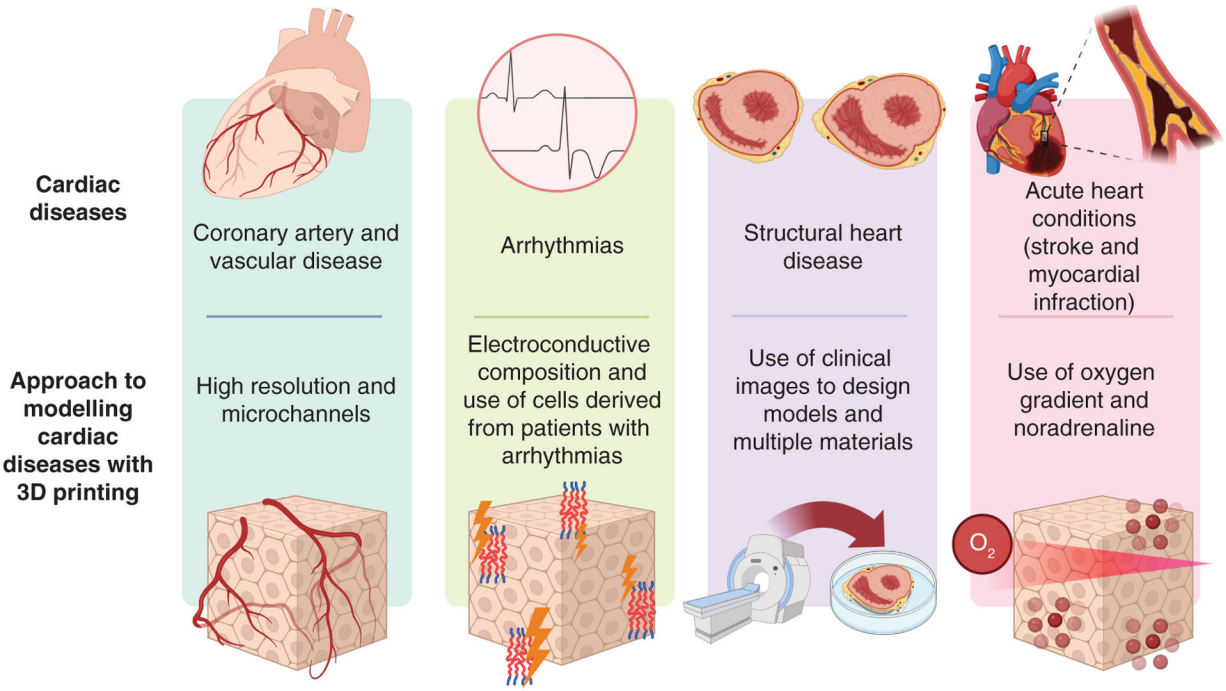

Figure 3.

How cardiac diseases (vascular, arrhythmia, structural and acute conditions) can be modeled using 3D bioprinting.

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Table 1.

Outlining the different model designs, materials, cell types, printing methods and functionalities of all 3D bioprinting cardiac models discussed in this review.

Study	Model design	Materials	Cell type	Printing method	Functionality	Ref.
Xu *et al.*	Half heart with a 1 cm inner diameter and two connected ventricles	Alginate/gelatin crosslinked with calcium chloride	Mammalian cardiomyocytes	Inkjet-based bioprinting	Microscopic and macroscopic contractile function *in vitro*	[62]
Wang *et al.*	Constructs either string form or patch form; entire construct was 1.8 × 1.6 cm^2 and 0.6 mm thick	Fibrin, gelatin, aprotinin, glycerol, and hyaluronic acid; a sacrificial hydro gel of gelatin, glycerol and hyaluronic acid used to support the cell-laden hydrogel while printing	Cardiomyocytes from infant rat hearts	Extrusion-based bioprinting	Spontaneous synchronous contraction; cardiac tissues were formed with electromechanically coupled cardiac cells	[65]
Zhang *et al.*	3D microfibrous scaffolds with anisotropic arrangements (crosshatch pattern)	Alginate and gelatin methacryloyl	Human umbilical endothelial cells and neonatal rat cardiomyocytes	Extrusion-based bioprinting	Aligned endothelialized myocardium with spontaneous and synchronous contraction capabilities	[67]
Gaebel *et al.*	Human umbilical vein endothelial cells printed in two layers in an orthogonal grid pattern with a 90 μm grid-line distance, followed by mesenchymal cells in two layers	Polyester urethane urea	Human umbilical endothelial cells and human mesenchymal stem cells	Laser-based bioprinting	Observed vascular tube formation; functional improvement of infarcted hearts after transplantation in rats	[20]
Lu *et al.*	Symmetrical stent structure with wires forming a uniform diamond shape	Poly (p-dioxanone)and Stabaxol −1	Endothelial cells	Extrusion-based bioprinting	Good proliferation when endothelial cells were seeded into the bioresorbable stents	[11]
Park *et al.*	Patches with a thickness of 3 mm and diameter of 8 mm	Heart-derived extracellular matrix hydro gel	Bone marrow-derived mesenchymal stem cells	Extrusion-based bioprinting	High cell survival rate and significant improvements in cardiac function and vessel formation	[80]
Alonzo *et al.*	Annular ringlike scaffolds	Gelatin and alginate	Human cardiomyocytes, fibroblasts and microvascular endothelial cells	Extrusion-based bioprinting	Heterocellular cardiac cell interactions, paracrine signaling and cardiac contractions	[81]