Narrative Review Article

Comparing Recent 3D Bioprinting Methods and Bioinks in Replicating the Structural and Functional Complexity of Native Cardiac Tissue

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ABSTRACT

After cardiac injury, such as a heart attack, it is difficult for cardiomyocytes to regenerate. Recently, 3D bioprinting (3DBP) has become increasingly explored for its methods and bioinks to repair or restore damaged myocardium. 3DBP precisely places cells and biomaterials into cardiac architectures that aim to resemble the structural complexity of native cardiac architectures, as well as mimic the functionality, which includes levels of cell viability, electrical conductivity, vascularization, and modulus. There are several main methods of 3DBP, such as extrusion-based, which uses a nozzle, or stereolithography, which uses light. These methods vary in the regulation of other factors, including resolution, bioink viscosity, and multi-material compatibility. Furthermore, the materials chosen for bioinks directly determine cell interactions with one another and with the surrounding extracellular matrix, and are typically selected in accordance with the geometry of the targeted cardiac structure. Natural bioinks offer high biocompatibility; synthetic bioinks have improved printability and stiffness; and hybrid bioinks can provide the advantages of both, but come at the cost of more complex formulations. The purpose of this review is to provide a comprehensive overview of these methods and materials, and compare these in terms of their individual advantages when applied to the cardiac structure most compatible with their features. Overall, this information will be critical to aid researchers in developing novel cardiac tissue engineering treatments.

Keywords: cardiac tissue engineering; regenerative medicine; 3D bioprinting; 3D bioprinting methods; bioinks; bioink cell viability; bioink materials; bioink cell geometries

INTRODUCTION

Cardiovascular disease is the leading global cause of death worldwide, causing 32 percent of all deaths (1). Unfortunately, the adult human heart is the least regenerative organ in the body due to the limited regeneration capacity of cardiomyocytes, the major cell type in cardiac tissue. This leads to minimal tissue repair in the heart after injury, which can significantly impair cardiovascular function (2). Thus, there is a need to improve cardiac tissue regeneration following injury to restore cardiac function.

Tissue engineering is a new field of research that aims to recreate living tissues to replace damaged or diseased ones. As such, it is a promising approach for repairing damaged cardiac tissue. 3D bioprinting (3DBP) is a common approach to generate functional tissues with

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specific geometries, mimicking the biological structure of the heart and the surrounding vasculature. The choice of the 3DBP method as well as the bioink utilized could impact the overall therapeutic outcome, requiring careful selection and optimization for successful cardiac-related 3DBP.

The purpose of this review is to compare recent advances in 3DBP in replicating the structural and functional complexity of native cardiac tissue. Briefly, the background of cardiac tissue and cell composition will be discussed, followed by a background of 3DBP and its major methods, and then an in-depth comparison of the methods. Finally, a future outlook of the ideal methods that can be applied to different cardiac tissue injury scenarios with the goal of aiding in the selection of 3DBP methods for future cardiac tissue engineering therapeutics will conclude the review.

BACKGROUND OF CARDIAC TISSUE AND THE CLINICAL NEED FOR CARDIAC ENGINEERING

Cardiac structure, tissue organization, and cell composition

Cardiac tissue facilitates the involuntary rhythmic contraction of the heart. This fatigue-resistant muscle ensures consistent circulation and maintains blood pressure. The ventricular walls of the heart are composed of three layers: outer epicardium, middle myocardium, and inner endocardium (3). The myocardium (e.g., the middle and thickest layer of the heart) (2) is primarily composed of cardiomyocytes. Cardiomyocytes are the specialized muscle cells that are electrically stimulated to maintain the normal physiological processes of the heart. They make up ~75% of the volume of the myocardium and monitor a sophisticated interplay of muscle contractions, electrical signals, and metabolic regulation (4). In addition to cardiomyocytes, cardiac fibroblasts are non-contractile structural cells that create and repair various components of the extracellular matrix (ECM); this determines the stiffness, flexibility, signaling, and contractility in the heart muscle (2). Specifically, the myocardial ECM is a network of proteins, primarily collagen and elastin, which provides essential scaffolding, stability, and signaling for cardiomyocytes to perform active contraction.

Cardiovascular injuries and cellular impact

There are several types of cardiovascular injuries that can occur. For instance, a cerebrovascular accident, or stroke, is the result of disrupted blood flow to the brain. Aortic dissection is the weakening or tearing of the aorta (5). In this review, myocardial infarction (heart attack) results from a vascular blockage that prevents blood flow to the cardiac tissue, often resulting in a non-contractile scar (6). Cardiomyocytes are damaged or die, primarily due to oxygen and nutrient deprivation (ischemia) or inflammation from blockages, spasms, or high demand (2). These injuries can permanently damage cardiac tissue and vasculature, highlighting the critical need for regenerative medicine.

These injuries can significantly impact the myocardium and impair cardiac function. For example, increasing preload, or the maximum stretching of cardiomyocytes, directly affects stroke volume and cardiac output. When excessive, it is a key factor in the development of heart failure (7). Disruption of anisotropy (materials exhibiting different characteristics depending on the direction of measurement) also impacts the heart's ability to generate torsion (the heart's natural twisting motion during a heartbeat to pump blood efficiently). Cardiac fiber rearrangement is associated with aging, along with sub-endocardial fiber rearrangement. The fiber orientation between the endocardial and epicardial surfaces becomes increasingly misaligned, making it challenging to attain the balance necessary for optimal torque generation (8).

Current treatment for heart failure and many of these cardiovascular injuries involves lifestyle changes, medications, devices (pacemakers, left ventricular assist devices), and invasive surgery (9). These methods have many complications, costs, and restrictions, and mainly just relieve symptoms and slow disease progression. Importantly, these treatments do not regenerate the myocardium. For advanced cases of cardiovascular diseases, a heart transplant is the only viable solution, but is constrained by limited organ donations and side effects from the immune system (2) (Figure 1).

3D CARDIAC TISSUE BIOPRINTING METHODS

Engineered cardiac tissues have to be designed with characteristic features of the myocardium, such as anisotropic cell orientations (cells aligning along specific axes), synchronized contractions, and exchange of nutrients. Using 2D surfaces is impossible to replicate the anisotropic architecture of native myocardium, whereas using 3D allows for spatially controlled engineering methods (4). 3DBP is an advanced approach for cardiac

engineering that can achieve these design parameters.

3DBP restores function by replicating cardiac architecture, electromechanics, and vascular support (10). It is a newly emerging biofabrication technology that allows spatially constructed living cells and biomaterials to construct functional tissues in vitro by combining cardiomyocytes with supporting cells such as fibroblasts, endothelial cells, and smooth muscle cells. To replicate extracellular environments, bioinks have been engineered by deriving natural biomaterials such as collagen, decellularized matrix, and synthetic polymers (11), which will be discussed in detail in the section "3D cardiac bioprinting bioinks." In this section, the major methods used for 3DBP will be discussed, where Figure 2 provides schematics for each method and Table 1 details specific parameters and limitations of each method.

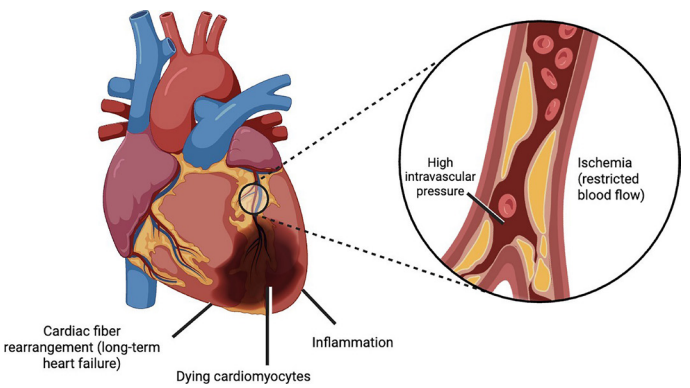

Figure 1. *Diagram of mechanisms of myocardial infarction. Ischemia and high intravascular pressure stemming from a coronary artery blockage lead to cardiomyocyte death within the myocardium. To compensate for the impaired cardiac function, there is inflammation and cardiomyocyte stretching, elevating internal pressure. Additionally, to compensate for the non-contractile scar after a heart attack, healthy muscle fibers rearrange to maintain contractility. As a result, the heart wall becomes thinner and more spherical, leading to long-term heart failure.*

Major 3D bioprinting methods

Extrusion-Based Bioprinting

The most common 3DBP technique in tissue engineering is extrusion-based bioprinting, which uses a nozzle to extrude the bioink in a layer-by-layer manner, accumulating into a 3D construct. There are different extrusion mechanisms to account for various specifications: pneumatic-based (pressurized air), piston-based (mechanically pushing for better control of bioink flow), and screw-based (mechanically pushing for better spatial control and high viscosity bioink release) (12). However, extrusion-based bioprinting is limited by low resolution, making it difficult to print complex, high-resolution tissue.

Inkjet Bioprinting

Inkjet bioprinting is another common 3DBP technique. This method generates droplets via thermal systems (≈20–100 µm diameter) or piezoelectric systems (≈30–100 µm diameter). These small droplet volumes allow for high

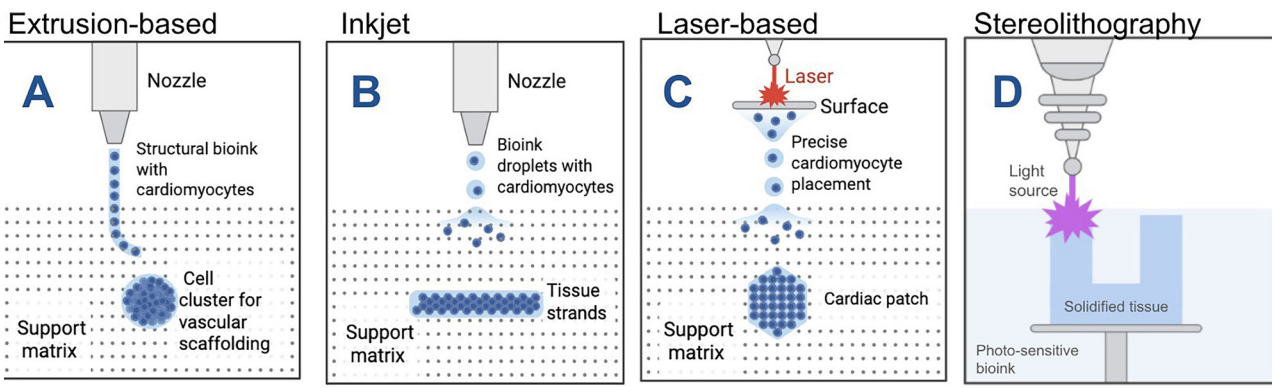

Figure 2. *A schematic showing the four major 3D bioprinting techniques. A) Extrusion-based 3D bioprinting utilizes a nozzle to print structural bioinks containing cardiac cells for scaffold fabrication, effective for the "walls" or larger architecture of the heart. B) Inkjet 3D bioprinting precisely uses a nozzle to dot cardiac droplets into thin, functional tissues. C) Laser-assisted 3D bioprinting is good for high-resolution cell patterning, crucial for electrical alignment of heart tissue. D) Stereolithography uses high-resolution light printing to allow for the creation of intricate heart valves and tiny blood vessel networks.*

Table 1. *Comparisons of 3DBP methods focused on resolution, precision, viscosity, and other parameters.*

Method type	Resolution	Cell placement precision	Cell viability	Bioink viscosity limits	Time frame	Shear stress damage	Multi-material capability	Targeted Structures
Extrusion	Medium: 100-300 μm	Medium	70–95%	High: 10^8 mPa·s	Slow (layer by layer)	High: 1–20 kPa	High: multi-head	Bulk tissue, thick strands, scaffold, large patches, organ-sized constructs
Inkjet	High: 10–80 μm	High	80–95%	Low: 1–40 mPa·s	Fast for 2D, slow for 3D	Medium: ≤5 kPa	Medium: multi-nozzle, but low-viscosity limit	Thin layers, patches, small constructs with high patterning resolution
LAB	High: 10–50 μm	High	90–98%	Medium: 300 mPa·s	Medium	Low: <1–3 kPa	Medium: switching is complex	High resolution cell patterns, microtissues, fine structures
SLA	High: 10–50 μm	High	80–95%	Medium: 100–200 0 mPa·s	Fast	Low: 0.01–0.1 kPa	Low: usually one resin at a time	Complex 3D scaffolds with fine internal channels and networks

resolution and precise, programmable cell placement. However, low-viscosity bioinks are required for reliable droplet formation, which narrows material choices for cardiac tissue. Additionally, nozzle clogging and creating uniform droplets still remain a limitation (10).

Laser-Assisted Bioprinting

A less common bioprinting technique is laser-assisted bioprinting (LAB) due to its complexity and high cost. Instead of a nozzle, a laser beam projects onto a donor slide—covered with the bioink—and causes a jet of the bioink to deposit onto the receiver slide, hereby printing. LAB must have mid-range viscosity bioink, and printing speeds higher than 200 m/s can be dangerous to cells. Still, LAB is advantageous because of its high cell viability and cell density, and it eliminates the issue of clogged nozzles (12).

Stereolithography

Stereolithography (SLA) is a higher resolution, nozzle-free bioprinting method that uses a light projector to solidify the bioink layer-by-layer. It has recently been used to create entangled vascular networks in the alveolar wall in the lungs (4). SLA has also been used to print cell-laden hydrogel patches to the heart as a therapeutic approach for myocardial infarction. Additionally, SLA has fabricated abnormally narrow aortic valve models to study blood flow and pressure changes in the heart (13). In SLA, the photo-sensitive bioink undergoes a photocuring process, which traces a defined pattern that crosslinks to form the final microstructure (12). Limitations of SLA include high equipment costs and messy post-processing of UV curing, and materials are limited to more brittle photopolymers.

Bioprinting Method Effects on Cell Viability

Extrusion-Based Bioprinting

For extrusion-based 3DBP, cell toxicity correlates with the high shear and pressure in the nozzle. If the viscosity is high or the nozzle is too narrow, the increased force causes viability to drop, particularly for more fragile cardiomyocytes. Extrusion parameters surrounding viscosity are implemented to avoid shear-induced death of the cells, such as extrusion pressure and using conical nozzles instead of cylindrical nozzles (3). Extrusion bioprinting can keep cell viability high when nozzle shear stress is kept below ~5 kPa, but viability drops to ~76% or less when shear exceeds ~10 kPa, especially

for sensitive cells (1). To support very fragile human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) in extrusion printing, one group extruded them in a collagen-hyaluronic acid bioink to improve printability. This way, they could accurately print tissues that remained spontaneously contracting for months (14).

Inkjet Bioprinting

Inkjet 3DBP offers high resolution (≈20-100 μm) and fast droplet deposition, but bioinks must be very low viscosity (~3-10 mPa·s) and have low mechanical stress to form droplets (12). This limits how many cells, bioink, and matrix choices can be included. Moreover, the droplet formation exposes cells to repeated mechanical and sometimes thermal pulses. To preserve viability, viscosity, cell density, and droplet-generation conditions must be kept in a safe range to control toxicity (9). So, inkjet 3DBP is less ideal for dense, fragile cardiac tissues, which require high hiPSC-CM loading and a more protective ECM (12).

Laser-Assisted Bioprinting

In LAB, cells experience heating and mechanical shock from the laser pulse, so excessive energy or high ejection speeds should be avoided to prevent damaging cell membranes. Still, LAB is nozzle-free and can handle highly viscous bioinks (1-300 mPa·s) while still achieving high resolution and overall high cell viability. LAB is especially suitable for fragile or rare cells that shouldn't be forced through a narrow needle, provided that UV/laser exposure is kept low enough to avoid DNA and protein damage (1).

Stereolithography

For SLA, the main toxicity risks are UV, or visible light exposure, and photoinitiators. High-intensity UV can damage DNA (3), enzymes, and protein denaturation, which is especially problematic for delicate stem-cell-derived cardiomyocytes (1). SLA bioinks must be formulated with milder photoinitiators, lower light doses, or longer wavelengths to balance cell safety (3). For fragile cardiac cells, SLA is typically paired with softer, ECM-like hydrogels, and is used more for high-resolution patterning of support scaffolds rather than for very high-density direct cell encapsulation (11).

3D bioprinting in cardiac applications

3DBP in cardiac tissue engineering has fabricated architecturally complex and functioning cardiac structures, with alignment from the cellular level to valves and ventricles at the organ scale (15). It is able to spatially control cells and living biomaterials to construct precise and functional cardiovascular tissue.

Imaging data and computer-aided design address the dual challenges of replicating myocardial contractility and perfusable vascular networks (11). The initial goal was to create replicas of cardiac tissue to repair or replace damaged myocardium, and today, the main obstacles to this approach have emerged. To produce a reliable resemblance of 3D cardiac tissue, it is required that there is: 1) a large number of cardiac cells for physiological density, 2) biocompatible scaffolds, and 3) biomimetic signaling systems, which include mechanical and electrical signals (2).

In one study, Enayati *et al*. fabricated a 3D-printed patch made from polycaprolactone and coated with human ECM. This patch enabled attachment and proliferation of various cardiac cells compared to non-ECM-coated patches. Wu *et al*. developed a 3D printed, multi-layered scaffold using the polymer polycaprolactone (PCL) to mimic the natural, multi-layered structure of cardiac tissue. Specifically, when induced pluripotent stem cells were cultured on this scaffold, it was observed that cells aligned with the printed fibers and had greater expression of ECM-related genes. When put into the hearts of mice following myocardial infarction, this scaffold increased angiogenesis in the heart and improved cardiac function.

3D CARDIAC TISSUE BIOPRINTING BIOINKS

Overview of Bioink Features and Materials

Bioinks are the keystone of 3DBP in cardiac tissue engineering. On top of precision and structural stability, bioinks also influence long-term cell viability, tissue maturation, and function. The chosen material directly determines cell interactions with each other and their surrounding ECM. If the bioink is too soft, it deforms easily and dissipates contractile forces instead of transmitting them between cardiomyocytes. If it is too stiff, cells are restricted from spreading and migration, leading to reduced ability for cardiomyocytes to shorten and contract (10). Bioinks have been categorized into three primary groups: natural bioinks (ECM components and biopolymers), synthetic bioinks (synthetic polymers), and hybrid bioinks (combine advantageous properties of both natural and synthetic materials) (11). Below, the details for each type of bioink will be reviewed, along with key examples.

Table 2. Comparisons of 3DBP bioink materials, focused on viability, degradation, applications, and other parameters. *Note: dECM = decellularized extracellular matrix; PU = polyurethane; PEGDA = polyethylene glycol diacrylate; GelMA = gelatin methacryloyl.*

Materials	Modulus	Viscosity	Cell viability	Toxicity	Degradation	Common 3DBP Method(s) Utilized	Applications
Natural Materials							
dECM	Low: 0.04–1 kPa	Low: 10–10^3 mPa·s	>85–95 %	Low when properly decellularized	Medium: days-weeks	Extrusion	Tissue-specific constructs, regenerative patches for ECM composition and signaling
Gelatin	Low: 0.5–20 kPa	Medium: 10^2–10^4 mPa·s	>85–95 %	Low when diminishing photoinitiators and UV risks	Medium: days-weeks	Extrusion and light-based	Cell-laden constructs, vascularized tissues
Fibrin	Low: 0.1–5 kPa	Low: 1–10^3 mPa·s	>90%	Low because naturally wound-healing	Fast: days-weeks	Extrusion, some inkjet	Vascular constructs, wound-healing and skin, patches
Synthetic Materials							
PEGDA	Low: 0.1–1 kPa	Medium: 10^2-10^3 mPa·s	70–90%	Medium: requires good formulation and post-curing rinses	Slow: weeks-months	SLA and other light-based, also extrusion	Scaffolds for myocardial tissue, soft tissue, structural supports
PCL	High: 10^2-10^3 MPa	High: >10^4 mPa·s	>90%	Low because generally biocompatible	Slow: months-years	Melt-extrusion	Scaffolds for cartilage and bone, frameworks, structural supports
PU	Medium: 0.1–10 MPa	High: >10^4 mPa·s	90–98%	Medium: needs to be fully cured and cleaned	Medium: variable; weeks-years depending on construct	Melt-extrusion	Soft tissue scaffolds, elastic ligaments, flexible implants
Hybrid Materials							
dECM-PE GDA	Medium: 1–50 kPa	Medium: 10^2–10^3 mPa·s	80–95%	Low: main concerns are with PEGDA acrylates	Medium: weeks	Extrusion and SLA	Cardiac, cartilage, liver, and skin where dECM provides biochemical cues and PEGDA provides stiffness
Alginate-GelMA	Medium: 5–100 kPa	Medium: 10^2–10^4 mPa·s	80–95%	Medium: risks with photoinitiator and UV for GelMA	Medium: days-weeks	Extrusion, also SLA	Cartilage and bone scaffolds, vascularized tissue, cell-laden constructs

Natural Bioinks

Natural bioinks are highly biocompatible but harder to control mechanically and reproducibly than synthetic or hybrid systems. But overall, they are considered superior to synthetic materials since they can use native ECM molecules to replicate foreign-body responses at the region between the implanted biomedical material and the living tissue (16). Natural bioinks can stimulate cell proliferation, guide migration, and activate regulatory signaling pathways relevant to cardiac differentiation (17). Using natural bioinks can still be a challenge because ECM-based biopolymers are often soft, affecting scalability and structural fidelity. Also, large-scale production is difficult because of source tissue variability and lack of reproducibility and standardization (16).

Heart-derived decellularized extracellular matrix (dECM) is a standout natural material because it contains a complex mix of proteins, glycosaminoglycans (tissue repair and remodeling), and growth factors. Thus, dECM better mimics an environment for tissue-level functions. Its high concentration is a challenge for printing, affecting scalability and structure, so combating methods include dual crosslinking and adjustable elasticity (9), similar to polyethylene glycol diacrylate (PEGDA)'s methods (see below). It is shown that in dECM bioink, human cardiac fibroblasts survived extrusion and photopolymerization with >97% viability after 7 days, showing high cytocompatibility for heart-relevant cells (16).

The major constituent of the ECM is collagen. It is considered an excellent cell delivery platform for cardiac applications because it naturally promotes cardiomyocyte adhesion and differentiation, and is also more available to labs than dECM. Gelatin is frequently used as a printable hydrogel for neonatal cardiomyocytes. In one example, gelatin microchannels were shown to orient stem cells and improve the organization and rhythmic beating of cardiomyocytes. Gelatin methacryloyl (GelMA), made from chemically modifying gelatin, serves as a scaffold to control cell shape, orientation, and well-aligned myofiber patterns that generate high contractile force (3).

Finally, a range of other natural materials is starting to be utilized in 3DBP for cardiac engineering. The common natural polymer fibrin is typically bioprinted with iPSC (induced pluripotent stem cell)-cardiomyocytes and endothelial cells. Alginate is often combined with fibrin to create fibrin-alginate composite hydrogels; these hydrogels have a crosslinking interstrand distance, which increases stiffness and porosity (3). However, alginate has low electrical conductivity, requiring the addition of fillers.

Furthermore, hyaluronic acid and other ECM components are commonly used alongside these materials (18).

Synthetic Bioinks

For synthetic bioinks, synthetic-ECM parts can improve printability and stiffness for fibroblasts and cardiomyocytes. Synthetic bioinks are more stable and reproducible due to their high viscosity and long-term mechanical strength, and are manufactured under controlled conditions. Rather than decellularized ECM, which can vary with source tissue and processing (19). Still, synthetic bioinks are more cytotoxic (the quality of being toxic to cells) because they do not provide the complex ligand and growth factors of the ECM. They often need added peptides, proteins, or hybridization with ECM, such as collagen and gelatin, to support cell adhesion and maturation. Consequently, they require adhesion motifs for good spreading and coupling (20).

Researchers have created bioinks with adjustable stiffness simply by changing the crosslinking conditions to mimic healthy and fibrotic heart tissue (9). PEGDA, one of the more popular synthetic polymers, is a polymer used as a crosslinker to increase stiffness, as well as improve mechanical strength and printability while still keeping cells alive. PEGDA is important for building thicker or more complex cardiac constructs. Recent development of bioink reviews points to methacrylated synthetic hydrogels, which include PEGDA, as key ingredients in high-resolution bioprinting methods since they can be rapidly crosslinked, particularly by light in SLA methods.

Another common synthetic bioink material is PCL, a flexible, strong polyester usually used as a scaffold in heart and vessel constructs due to its good mechanical strength and slow biodegradation. PCL can also be printed as supportive lattices that are then combined with softer, cell-laden hydrogels. It is used like a skeleton that can handle repeated, compressed loading, while softer cell-compatible gels carry the biology (11). Similarly, polylactic acid (PLA) and poly(lactic-co-glycolic acid) (PLGA) are also used as structural matrices or reinforcement in cardiac patches and scaffolds, due to their tunable stiffness and degradation rates. They give a higher modulus, but need to be combined with ECM-like components to ensure survival of cardiomyocytes and vascular cells (21).

Polyurethane (PU) is an important elastomer in heart applications because of its high elasticity and fatigue resistance. It is composed of "soft" segments (flexible

polyester chains) and "hard" segments (urethane groups with strong hydrogen bonds). The soft segments stretch and recoil while the hard segments are reversible physical crosslinks, giving PU its elasticity under the repeated strain of the beating heart. PU is suitable for repeatedly contracting cardiac patches and valves or vascular scaffolds (21).

Poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) is a conductive polymer that is incorporated into hydrogels and bioinks to increase electrical conductivity, improve synchronous contraction, and couple cardiomyocytes in engineered heart tissues. When dispersed in hydrogels, it creates conductive pathways that lower electrical resistance. However, the polymer's synthetic, charged nature means that its concentration and distribution must be controlled to avoid local toxicity or stiffness mismatches (11).

Hybrid Bioinks

Hybrid cardiac bioinks try to combine the bioactivity of natural ECM with the mechanical and printing control of synthetics, but still have trade-offs to attain this. In cardiovascular bioprinting, alginate is often blended with GelMA to form hybrid bioinks that enhance cytocompatibility, particularly to print perfusable vascular channels and tubular constructs. PEGDA is another key synthetic component combined with gelatin or dECM to improve cell viability and function while retaining stiffness.

PEGDA-based hybrid bioinks can be precisely tailored to mimic mechanical environments on a range of stiffness (11). Hence, dECM-PEGDA hybrid bioinks have demonstrated >94-97% viability of encapsulated human cardiac fibroblasts and hiPSC-cardiomyocytes (22). It is known that combining natural polymers (gelatin, alginate, hyaluronic acid, etc.) with synthetic polymers like PEG or PCL improves extrudability and structural integrity over just those components alone (23). Hybrid bioinks have also been shown to significantly improve electrical conductivity and cardiomyocyte coupling (11).

However, hybrid bioinks require more complex formulation and careful tuning of mixing ratios, crosslinking, and rheology (flow of matter) due to the fact that PEGDA or other synthetics can easily overshoot stiffness (23). Excessive PEGDA can also reduce gel toughness, increase the potential for synthetic-related cytotoxicity and inflammation (24), dilute native ECM components, and reduce regenerative signaling advantages that come from dECM (19).

Bioink Material Effect on Cell Viability

As previously mentioned in the section "Background of Tissue Engineering, 3D bioprinting, and its role in cardiac tissue engineering," cardiac tissue engineering focuses on combining cardiomyocytes with supporting cells such as fibroblasts, endothelial cells, and smooth muscle cells to create the heart's multicellular structure with electrical connectivity and contractile function. As such, four dominant cell types in the myocardium—cardiomyocytes, cardiac fibroblasts, smooth muscle cells, and endothelial cells—are used as bioinks for cardiac constructs, which include alginate, collagen, gelatin, hyaluronic acid, and decellularized ECM (12).

Natural Bioinks

Natural bioinks (e.g., dECM, collagen, fibrin) generally have high biocompatibility and native adhesion to support high viability and long-term function, but they cannot be too soft or weakly crosslinked (16). Although dECM-based bioinks retain native ECM molecules and allow cell recognition and attachment without provoking immune responses, which is favored for long-term viability, pure dECM often has a low modulus and poor shape fidelity. High concentrations or extra crosslinking are needed, making constructs denser and mechanically less optimal for cell spreading and diffusion (25).

Synthetic Bioinks

Synthetic polymers can be tuned to match a desired elastic modulus, but higher photoinitiator concentrations increase crosslinking and stiffness. This comes at the cost of a proportional decrease in cell viability, showing how aggressive synthetic crosslinking can harm embedded cells. With high stiffness, encapsulated cells cannot spread or remodel the matrix, impairing survival. Contrastingly, soft gels may collapse and concentrate cells, also harming viability in the long run. With synthetic systems, high viscosity combined with small nozzles pushes shear stress above ~10 kPa, where average viability can drop to around 70-76% viability, especially in extrusion-based printing (1).

Hybrid Bioinks

For hybrid bioinks, cardiac dECM-PEGDA hydrogels use PEGDA photopolymerization after printing to raise the modulus while still maintaining >97% viability of encapsulated human cardiac fibroblasts at 7 days. This shows that hybrids can improve mechanical strength without sacrificing cytocompatibility or viability (16). As already mentioned, dECM has a low modulus and

poor printability. This can cause sagging and shape loss. Adding synthetic crosslinkers (e.g., PEGDA, methacrylated polymers) improves viscosity, print fidelity, and mechanical stability, indirectly supporting better viability in thicker or more complex cardiac constructs (25). Additionally, too much synthetic polymer or too strong crosslinking can reduce gel toughness or create cytotoxic degradation, increasing inflammation and undermining the biological advantages of the ECM component (19). Therefore, hybrid designs require optimization of the natural-to-synthetic ratio, photoinitiator level, and crosslinking time to keep viability high while achieving target cardiac stiffness and geometry (23).

Bioink Cell Geometries

Bioink materials set basic properties like viscosity, stiffness, elasticity, degradability, and bioactivity, which in turn decide which geometry can be realistically printed and kept alive. The main geometries are soft cell-dense slabs, stiff tubes, and stable lattices.

Natural Bioinks

Natural bioinks have great biology, but limited self-support. Their main advantage is high cell viability over extreme structural complexity. Because of their softness and weak crosslinks (brain/liver-like, <1-10 kPa), they have low stiffness and yield stress (26), so they are more optimized for sheets and supported patterns instead of tall, free-standing valve tubes or beams. They can spread or sag once deposited, so printing thin patches, short crosshatch meshes, or slab-like tissues on a support surface is more reliable. A long tube printed out of pure dECM or collagen would likely deform under its own weight, not long after crosslinking (27).

Additionally, many ECM-like gels allow continuous filament extrusion, enabling defined lines, grins, and thin-walled tubes instead of droplets or blobs (28). Due to the high viscosity of natural bioinks, thermoresponsive ones (e.g. gelatin, GelMA) can be printed near their gel point to hold filament geometries to allow for small bridges or lattice scaffolds—however, the length is limited, and supports are needed (29). Ultimately, keeping printing in a moderate window below 10^4 mPa·s allows extrusion of fine, uniform filaments without clogging, which is crucial for high-resolution channels and small-diameter tubes (28).

Natural bioinks are also often fast-degrading. Ones like unmodified gelatin and fibrin are suitable for temporary patches or provisional scaffolds. To resist early collapse or to hold complex tube and scaffold geometries for weeks, natural networks must be chemically modified to enable thicker walls and nodes. Degradation-driven increases in porosity can be used intentionally. Scaffolds are often printed with smaller initial pores if long-term enlargement is expected for cell ingrowth (30).

Since natural bioinks contain adhesion motifs that promote spreading, their geometries often include open lattices and thin patches that allow cells to fully migrate (29). Additionally, high cell density in natural matrices leads to strong contractile forces (26).

Synthetic Bioinks

Synthetic bioinks allow precise modulus control ranging from very soft to cartilage-like stiffness. This enables architecturally complex, tall scaffolds and long-span beams that can be difficult with softer natural gels. Stiff synthetic frames are often used as structural "exoskeletons" that are filled or coated with more cell-friendly materials (31). Another advantage is predictable shear-thinning and yield stress. However, high viscosity synthetic bioinks require higher pressures that may limit printing with very delicate cells, so encapsulated cell viability can be lower unless formulation and process are carefully optimized (32). Additionally, since synthetic constructs have limited bioactivity, cells may remain poorly spread, so geometries often allocate dedicated "cell zones" or co-printed natural layers for viable cell niches (33).

Synthetic bioinks usually degrade more predictably than natural ones, making them suitable for long-term load-bearing scaffolds where architecture and walls must remain stable for months, which is critical for vascular grafts or bone support structures. They can also resist enzymatic degradation better than natural gels, so they are often used as outer shells or reinforcements around more degradable centers (33). In some implants, synthetic scaffolds are even designed to be non-degradable and permanent (31).

They also often have to be functionalized with adhesion ligands and growth factor-binding domains to support cell attachment and cell behavior. Similarly, they also frequently serve as carriers for controlled release of natural materials or peptides, with release aligned to specific scaffold zones or channels (31). Synthetic structures can also be more compact and efficient, since their qualities like degradability, bioactivity, and stiffness are highly tunable, so they can be integrated into a single printed geometry.

However, the lack of bioactivity that needs to be

replaced by natural materials can make printing more complex and limited.

Hybrid Bioinks

Hybrid bioinks combine natural polymers for bioactivity with synthetic networks for mechanical strength, to create cell-friendly environments within self-supporting scaffolds and tubes. These formulations can also reach higher toughness than natural inks while also maintaining ductility. As a result, hybrids can enable more complex 3D geometries by achieving shear-thinning behavior that supports multi-layer printing (32). They are also quite durable, with properly designed hybrids being able to print through small nozzles while maintaining viability (30).

Hybrids are often chosen for constructs where cells must remain viable in mechanically robust regions, such as bone-mimetic scaffolds with high stiffness and embedded stem cells (34). The natural components still facilitate post-printing remodeling as normal, allowing cell-driven refinement of geometry even within stiff frameworks. Degrading features take advantage of both synthetic and natural, by slowly degrading synthetics as long-term supports and faster-grading naturals for more temporary cell niches (21). For instance, in bone and cartilage applications, synthetic scaffolds maintain shape while natural components degrade to make space for mineralized ECM (34).

Natural ECM-derived biochemical cues (e.g., gelatin) can be exploited within synthetic frameworks to direct cell differentiation in specific zones of the scaffolds. This is shown with growth-factor-loaded natural microphases embedded in synthetic lattices that provide localized biochemical stimulation, like VEGF (vascular endothelial growth factor)-releasing regions in vascularized constructs (31). Overall, hybrid bioinks are favored for geometries where regions must differ in stiffness, degradation, and bioactivity within a single printed construct (30).

CONCLUSION

Cardiac engineering is a revolutionary way to engineer new cardiac tissue following various injuries. 3DBP is a promising approach to repairing or replacing damaged myocardium by utilizing the advantages given by various forms of bioprinting methods and bioinks. It enables spatially controlled reconstruction of cardiac architecture and vasculature, but no single method yet fully recreates the anisotropic, electrically coupled, and perfusable myocardium.

3D cardiac bioprinting methods

Extrusion bioprinting can handle cell-dense bioinks and build thick patches and scaffolds, but its lower resolution and high shear stress limit its applications for fine cardiac microstructures and fragile hiPSC-CMs. Inkjet bioprinting offers higher resolution and precise droplet placement. However, its low viscosity window and risk of nozzle clogging make it less suited for dense ECM-rich cardiac bioinks or thick myocardium-scale constructs. Laser-assisted bioprinting also provides high-precision cell placement with nozzle-free patterning, with excellent cell viability and high cell density. Yet, the main limitations include complexity, cost, and modest throughput that limit its application to large myocardial patches.

Stereolithography and related light-based methods have high structural resolution and can fabricate intricate valves and microvascular networks. However, SLA is dependent on photoinitiators, light toxicity, and brittle photopolymers, which constrain use with very fragile cardiac cells.

3D cardiac bioprinting bioinks

Natural bioinks such as dECM, gelatin, and fibrin provide bioactivity, cardiomyocyte adhesion, and signaling, which are their main advantages over synthetic bioinks. Natural bioinks are limited by their low modulus, variability, and limited shape fidelity, restricting their use to supported slabs, patches, and short tubes unless reinforced or chemically modified to become a hybrid bioink. Synthetic materials like PEGDA, PCL, and PU have highly tunable modulus, stability, and printability. They are mainly used for cardiac scaffolds, valve, or vessel frameworks. Moreover, materials like PEGDA are versatile and can be tuned for both scaffolding and soft tissue. However, biochemical functionalization depends on hybridization. Lastly, hybrid bioinks like dECM-PEGDA and alginate-GelMA balance the trade-off between the bioactivity of natural materials and the structural control of synthetic materials. Yet, hybrids demand careful optimization of natural-to-synthetic ratios, crosslinking, and rheology to avoid introducing synthetic-related cytotoxicity in ECM environments.

Limitations and future work

A key limitation across current cardiac bioprinting studies is the gap between in vitro function (organized beating patches and vascularization in small animals) and durable, electromechanically integrated human-

scale myocardium. This is due to constraints in vascularization, self-support, and the risk of abnormal heart rhythms due to faulty electrical impulses (32). Also, standardization is a limitation: there is variability in the dECM source, bioink formulation, and printing parameters that complicate direct comparison between studies. This slows advancement toward regulated, reproducible cardiac constructs.

Future work should focus on combining printing strategies that combine extrusion (for bulk geometry), SLA and other light-based methods (for microvasculature and valves), and inkjet or LAB (for high-precision patterning) to co-print myocardium, conduction pathways, and vasculature in a single integrated construct. Long-term clinical stimulation trials of printed cardiac tissues in animal models will be essential to understand maturation, immune response, and vascularization before moving toward human trials (34). Moreover, standardized printability metrics and open-source cardiac bioprinting platforms are needed to accelerate method and bioink comparison. The selection of bioink combinations for specific cardiac injury scenarios (e.g., epicardial patches vs valve repair vs ventricular wall reconstruction) will be more rational.

3DBP is also promising for pediatric cardiac care, in the sense that children born with congenital heart defects, such as septal defects, valve malformations, or any other abnormalities, undergo multiple repeated open-heart surgeries. Currently, treatments include synthetic patches or animal-derived tissue that need to be replaced. Bioprinted patient-specific patches based on a child's imaging data could better match native geometry and potentially be redesigned and reprinted as the child grows, reducing the need for multiple high-risk surgeries. Because many pediatric heart diseases are congenital and anatomically complex, high-resolution techniques (inkjet, LAB, SLA) combined with natural or hybrid cardiac bioinks could provide more accurate reconstruction of malformed valves, septa, or outflow tracts than current synthetic therapies. These recent advances in methods and bioinks for 3D cardiac bioprinting have been discussed and compared in this review, and future advances can hopefully lead to replicating the structural and functional complexity of native human cardiac tissue.

CONFLICT OF INTEREST

The author declares that there are no conflicts of interest related to this work.

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