Chapter 5
3D Bioprinting in Clinical Cardiovascular Medicine

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Abstract 3D bioprinting is a form of additive manufacturing tailored toward creating biological constructs with precise spatial control. As an extension of conventional 3D printing with a variety of materials such as polymers, ceramics, and metals, 3D bioprinting focuses on building viable, biomimetic products that can be used to replicate, improve, or substitute functional tissues. Driven by the field of tissue engineering, advancements in 3D bioprinting have enabled greater print resolutions, more customizable bioinks, and faster biomanufacturing speeds, which are critical when handling delicate biological substances. To date, researchers and engineers have creatively employed 3D bioprinting to combat cardiovascular disease, the most prevalent cause of death in the Western world. In the realm of cardiovascular medicine, 3D bioprinting has seen manifold applications including surgical models, cardiac patches, computational and theoretical models, heart valves, and stents. These technologies vary in terms of their extent of development, ranging

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from in vitro modeling to clinical therapies. While surgical models are most widely used in a clinical setting, other bioprinted models are rapidly developing with promising results. Overall, this chapter focuses on the clinical applications of 3D bioprinting aimed toward understanding, augmenting, or replacing cardiovascular tissues and organs.

Keywords 3D bioprinting · Additive manufacturing · Cardiovascular tissue engineering · Stents · Cardiac valve · Computational modeling · Surgical model

5.1 Introduction

3D bioprinting primarily aims for biomanufacturing of clinically applicable products that can replace diseased/damaged tissues or organs in vivo or creating biomimetic platforms to model various diseases in vitro. Clinical applications include a variety of direct regenerative approaches (e.g., printed tissue patches) and use as supplemental tools to improve current and future patient care methods (e.g., surgical models) (Fig. 5.1). Recent advances in bioprinting have made it possible to fabricate complex, patient-specific tissue architectures while maintaining the viability and function of multiple cell types that recapitulate the cellular and extracellular niche of the target organ/tissues [1]. While there remain some challenges for the clinical application of bioprinted constructs, development of new organ-specific

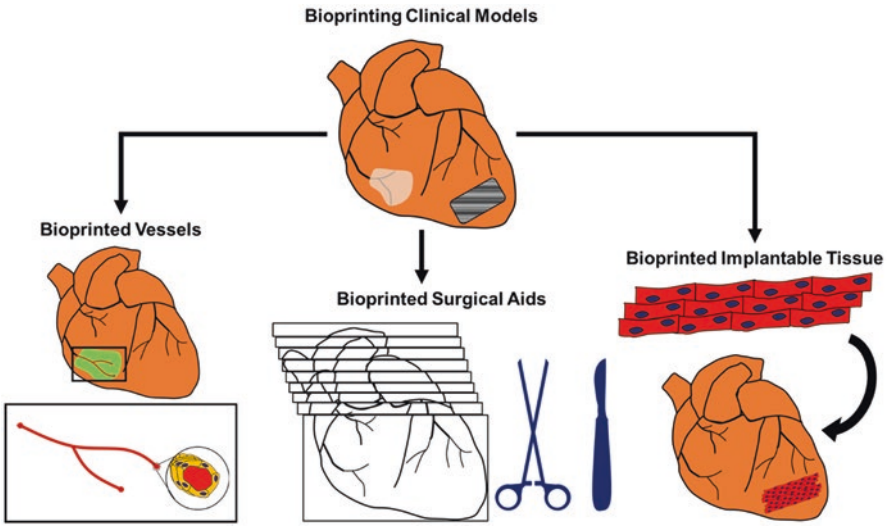

Fig. 5.1 Heart conditions can be addressed via bioprinting that can supplement surgical performance and aid in regenerative medicine. Bioprinted vessels can replace damaged or blocked native vasculature of the heart (left). Improved surgical aids can be developed via a combination of bioprinting and 3D printing approaches (middle). Bioprinted implantable tissue constructs (e.g., cardiac patches) can salvage heart structure and/or performance post infarct or with a congenital condition (right)

bioinks, state-of-the-art medical imaging technologies such as multi-contrast computed tomography (CT), and hybrid 3D bioprinting/printing approaches could be important steps toward translating bioprinting into a clinical setting.

To date, additive manufacturing, and in particular, 3D bioprinting have found rapidly growing applications in the fields of cardiovascular tissue engineering and regenerative medicine [2–5]. By providing a precise spatial control on the cell-biomaterial microenvironment, bioprinting enables recapitulating the complex physiomechanical, chemical, and biological cues of the native heart tissue [2, 6]. While the majority of cardiovascular tissue bioprinting efforts have been focused on restoring the anatomical and structural features of the tissues/organs, new research developments enable bioengineering of functional cardiac constructs [3, 7]. In addition to in vivo regenerative therapies, 3D printed products are increasingly used to enhance diagnosis and treatment of various cardiovascular diseases [8] (Fig. 5.1).

5.2 3D Bioprinted Surgical Models of Cardiac Disease

Bioprinting has grown from a strict research and development tool into a viable approach to generate surgical and clinical models of cardiac disease [6]. The major push that propelled bioprinting is the introduction of reliable, robust bioprinters and functional bioinks, which enable generation of a wide range or practical biomimetic constructs. Specifically, this technology is well suited to produce functional tissue, replacement vasculature for the heart, and high-fidelity anatomical models that can aid in surgical preparation and training [9–11]. Borrowing from the more established 3D printing field, bioprinting can specifically support the production of anatomical models to be used in cardiac surgery, such as surgical guides, templates, and stents (Fig. 5.2) [12]. Further, manufacturing implants such as tissue patches or replicates of the target area for direct organ repair are also possible via bioprinting [13]. Advantages of such 3D bioprinted tools and models include improvement of pre-operative planning, specifically enhancing the accuracy of the used techniques and available practice to perform complex surgeries prior to the operation. This can additionally save time in the operating room, increasing the odds for surgical success [14]. Still, several challenges remain that currently hamper widespread use of bioprinted surgical models and tools in the clinics. The accuracy of generated models is not always sufficient for the purpose, if their desirable characteristics are to be preserved. Depending on the mode of bioprinting, it can be a significant time commitment to generate an accurate biomimetic tool, which may be unfeasible for emergency surgery, though less of an issue when tackling chronic or diagnosed heart conditions. Finally, the relatively high costs associated with the hardware and software (bioprinters and professional CAD programs) and consumables (bioinks, cells, and molecules) is another limitation to routine use of bioprinting as a surgical aid at present [15].

Another application where bioprinting can be successfully translated into a surgical or clinical use is in the design of bioprinted tissue patches. Such printed tissue

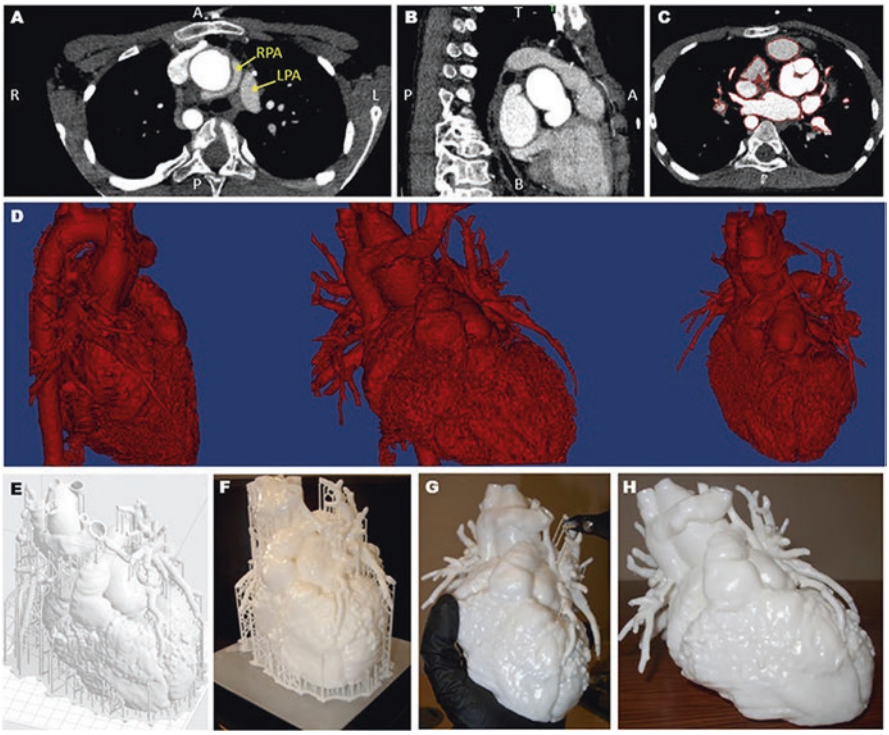

Fig. 5.2 Patient-specific model of heart segmented from computed tomography scans (**a–c**) into a 3D model (**d**). Sacrificial support scaffolds are generated (**e**), printed (**f**), and removed (**g**) to generate the finished heart model (**h**) [16]

models can be used as a surgical training tool, providing a safe, reproducible, and patient-specific platform on which novel devices or techniques could be tested. This could in turn help avoid further complications for patients or relying on an imperfect animal surrogate. Successful cardiovascular tissue models require tight controls over a range of physical parameters that allow to tailor a bioink to a specific clinical or surgical application. Bioink properties (e.g., viscosity, crosslinking mechanism, and resulting stiffness), mass transfer properties (e.g., diffusion and permeability), and functional modifications like biodegradability are some of the parameters that can be tuned to produce a faithful representation of the organ or tissue [17–20].

5.3 3D Bioprinted Cardiac Patches

With over 1.5 million cases of myocardial infarction (MI) each year, there is a demand for patient-specific heart tissue that aims to repair damaged regions [21]. A variety of approaches have been taken to produce cardiac patch devices [22–26].

The integration of human induced-pluripotent stem cell (hiPSC) technology has become a recognized modality for personalized heart tissue engineering [27–30]. Additional methods that are associated with manufacturing implantable cardiac patches are based on cells deriving from mesenchymal stem cells [31], secreted exosomes [32], and decellularized structures [33].

To date, a variety of 3D bioprinting approaches and a growing number of cardiac cell types are being used to manufacture functional cardiac patch systems (Fig. 5.3). A significant number of 3D bioprinted tissues employ cell-based therapeutic processes to improve cardiac function and salvage damaged tissue [34, 35]. Most of these engineered patches recognize the importance of non-muscle cells in myocardial structure and function, such as cardiac fibroblasts (FBs) and vascular cells [36]. Co-culture of cardiomyocytes (CMs) with endothelial cells (ECs), for example, takes strides toward implementing vascularization within constructed tissue architecture—a current focus in cardiac patch bioprinting [5, 37]. Other advantage of using multi-lineage cardiac cells, particularly during in vivo implantation, is the generation of natural tissue components (extracellular matrix or ECM) that are optimal for CM attachment and function [38]. Results from one study that performed in vivo implantation of bioprinted cardiac patches, composed of hiPSC-CMs and small proportions of human adult ventricular FBs and umbilical vein ECs, showed high cell density, but a lack of vascularization on the nude rat hearts [37]. A different experiment in which 3D bioprinted tissues with hiPSC-CMs, FBs, and ECs were tested, showed that maximum vascularization can be achieved by arranging the 3D fibers in a Janus geometry spatial organization [39]. Integration of vascularization in 3D bioprints opens new vistas of opportunities for translational application of cardiac patches with more biomimetic vascular networks [5, 40]. Cardiovascular tissue bioprinting is most commonly performed using naturally derived hydrogels as bioink. Synthetic bioinks are also being explored [41]. Naturally derived hydrogels offer cell viability and function, but are often associated with low resolution, poor handling, and inconsistency between batches. Alternatively, synthetic bioinks offer greater physical integrity and allows for more controlled physiochemical

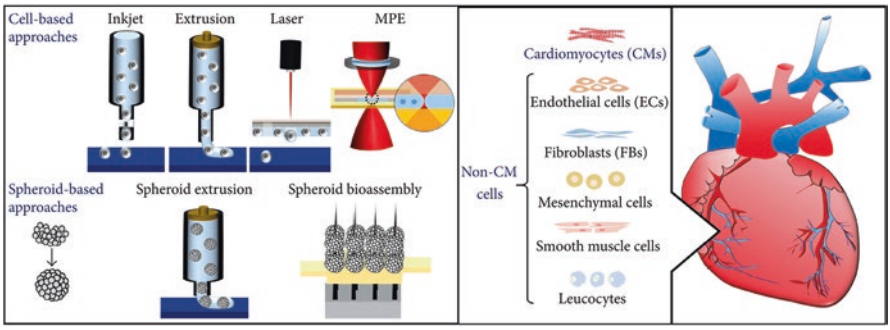

Fig. 5.3 Schematic illustration of various 3D bioprinting approaches used for manufacturing cardiac tissues (left) and specific cell types that reside in cardiac tissue (right) [42]

properties. Synthetic bioinks, however, may provide less accurate biomimicry, and insufficient support for cellular attachment, growth, and function [24].

Several studies have suggested that the distinct electrophysiological properties of nonhuman heart differ greatly from those of the human heart, causing a limitation that is inherent to most types of cardiac patch research [43]. Nevertheless, iPSC-CM-integrated cardiac patches, paired with pieces of decellularized heart ECM, have exhibited beating activity and electrophysiology comparable to those of the human heart muscle [38]. Other attempts to recapitulate human heart tissue in vitro include manufacturing (e.g., bioprinting) relatively larger and thicker human cardiac muscle patches (4 cm × 2 cm × 1.25 mm) [44]. The patch containing hiPSC-CM and hiPSC-smooth muscle cells (SMCs) demonstrated improved cardiac function and decreased infarct wall size and regional wall stress [44].

5.4 3D Bioprinting in Computational and Theoretical Modeling

3D printing provides a viable method for rapidly prototyping biomimetic fluid flow systems for physical flow visualization and computational fluid dynamics (CFD) validation. CFD models of cardiovascular fluids, primarily blood, have improved significantly in terms of their temporal and spatial resolution over the past few decades [45]. Improved imaging modalities, faster computing capabilities, and sleeker solving methods, among others, have enhanced the accuracy and relevance of CFD in clinical settings [46]. However, even the best analytical solvers need to be physically justified to ensure that the predictions made replicate real flow patterns, especially when turbulence is involved [47].

Cardiovascular applications of 3D printing have been studied in an array of diseased and surgically modified geometries. Variations in vessel cross-sectional surface areas in cerebral aneurysms [48], aortic stenosis [49], coarctations [50], and hepatic large vessels [51] have been investigated in normal and abnormal morphologies. Additionally, hemodynamic results of stents [52], embolic coils [53], and cavopulmonary connections [54] on neighboring flows are of significance since they not only study current effects of these corrective interventions, but also provide exploratory in vitro setups. These allow for earlier testing and a more detailed analysis of new devices. Overall, these studies demonstrated relative agreements between flow patterns of CFD simulations and physical models. As computing and imaging continue to improve, errors between these modalities dwindle.

In vitro replication of in vivo hemodynamic scenarios starts with medical imaging modalities such as CT [55], magnetic resonance imaging (MRI) [56], ultrasound and intravascular ultrasound (IVUS) especially for fetal heart monitoring [57, 58], and optical coherence tomography (OCT) [59]. Anatomical features are then segmented to stack 2D images into a 3D construct and meshed through software like 3D Slicer, Vascular Modeling Toolkit, or SimVascular. These files can then be 3D

printed or computationally modeled in a CFD software package (e.g., ABAQUS, FLUENT, HARVEY), which typically employs a finite element or lattice Boltzmann solver [45, 60]. Advantageously, the versatility of using the same 3D model to both physically and computationally model a patient-specific anatomical structure allows for consistency during comparison. To visualize fluid flows, many investigators choose to use particle image velocimetry (PIV) in their in vitro models, which captures videos of illuminated (fluorescent) microparticles (beads) and traces their local movement, providing excellent spatial resolution in cross section [54, 61]. Doppler ultrasonography [49] and 4D MRI [51, 62, 63] are also capable of measuring flow velocity, which can provide consistency if both patient and model are imaged with the same modality.

3D printing techniques differ and are typically optimized depending on desired material (e.g., flexibility or optical clarity), fabrication time, cost, resolution, and patient geometry complexity. Specifically, for CFD analyses, inkjet printing is the most common with printed accuracies up to 0.125 mm [48, 49, 52, 64]. Clearing scaffolding for print integrity can be challenging though [48]. Stereolithography (for stiff prints) [54] and laser sintering (for compliant constructs) [51] have provided similar fidelity of 0.1–0.15 mm, respectively. Lost wax molds have been used to generate urethane molds of high optical clarity that, when matched with a liquid of similar refractive index, show no appearance [50, 53]. Deposition extruded positive casts have served to create negative casts for tortuous flow geometries [61]. Both lost wax and deposition modalities can print within 0.2 mm of desired resolution.

While 3D printing has enabled high resolution replicates of clinical flow conduits for in vitro and CFD purposes, limitations exist. Due to the high computational demands of CFD, it is often regulated to specific regions, rather than a full-body vasculature. Not only printing microvasculatures of 1–10 μm, but reliably visualizing them is a challenge too. Since blood vessels are not simply passive vessels, replicating physiological flow mechanics is not trivial when accounting for blood rheology, pulsatility, and tissue compliance and active contractility [51]. Thus far, more traditional 3D printing has been applied for CFD applications, but similar applications of 3D bioprinting have yet to be augmented.

5.5 3D Bioprinted Heart Valves

Heart valve diseases are a serious dilemma worldwide with nearly 80,000 heart valve replacements occurring annually [6, 65]. Current treatment options are valve replacements with mechanical valves [66], bioprosthetic valves from porcine or bovine pericardium [67], or the Ross Procedure [68]. However, there are several downsides to each of these procedures. Mechanical valves often cause problems as a foreign body within the heart. Patients receiving these valves have to be on heavy medication for anticoagulation and immunosuppression for the rest of their lives [24]. Contrarily, bioprosthetic valves deteriorate over a shorter amount of time but can be placed less

invasively, reducing patient morbidity [67]. To improve valve longevity and functionality, tissue engineering approaches including decellularization, molded or structured scaffolding, electrospinning, or 3D bioprinting are being explored. Being able to 3D bioprint a biologically compatible heart valve would reduce time, costs, and risks that normally occur with a traditional valve replacement [69].

3D bioprinting allows for accurate replication of heart valves by scanning the 3D conduit, converting it to a STL file for post-processing and printing, and printing a cellular or acellular construct (Fig. 5.4). Bioprinting technology has greatly evolved over the past decade with new approaches allowing for manufacturing heart valves containing more cell types and biomaterials, as well as enabling more patient specificity [70, 71]. The biomimicry of engineered valves is rapidly improving, allowing for greater mechanical integrity, functional longevity, and cell viability [71]. In the future, bioprinting could create heart valves that are able to self-repair or grow as the patient's body grows [72]. The long-term goal for most heart valve tissue engineering studies is to create a valve that would be fully integrated with the host tissue to ensure long-term functionality [14]. Bioprinted valves are also being used in drug screening applications. For example, valve models, bioprinted using encapsulated human valvular interstitial cells (VICs) and exposed to osteogenic media, demonstrated enhanced micro-calcification. Such models can serve as an in vitro platform to study the pathogenesis of calcific aortic valve disease [70].

There are different hydrogels and biomaterials used for 3D printing of heart valves. Some commonly used bioinks include poly-ethylene glycol-diacrylate (PEG-DA), gelatin methacrylate (gelMA), and methacrylated hyaluronic acid (Me-

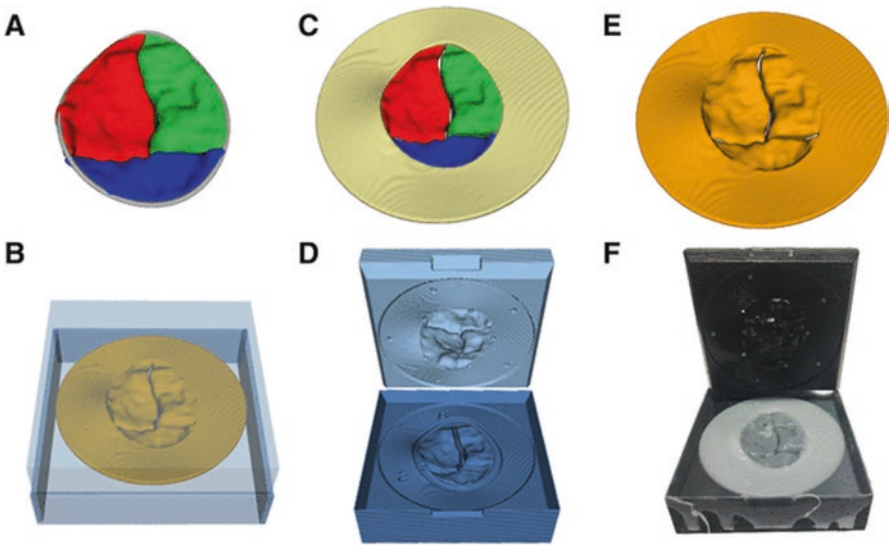

Fig. 5.4 Process of biomanufacturing mitral valve through direct 3D printing: segmentation (**a**), skirt addition (**b**), 3D model (**c**), template mold for 3D printing (**d**), final rendering (**e**), and printed valve within mold [73]

HA) [74, 75]. PEG-DA bioinks supplemented with alginate can be adjusted to have different concentrations of the material, leading to altered flexibility. This capability has been used to print both stiff and soft models. In particular, the stiff hydrogel is used to print the root wall of the valve and soft hydrogel is used to print the leaflets [75]. Results showed that bioprinting can create mechanically heterogeneous anatomical heart valve conduits. Major cell types that make up the human heart valve are SMCs, valvular endothelial cells (VECs), and VICS [65, 69]. Most studies use one of these cell types to determine the viability of the cells within the construct post-printing over desired periods of time. For example, porcine aortic VICs were cultured for up to 21 days in a construct made of a PEG-DA hydrogel with nearly 100% viability [75]. More recent findings show that anatomically complex, heterogeneously seeded constructs can be created using 3D bioprinting. This was done, for instance, using an alginate/gelatin bioink, printed with directly encapsulated human SMCs in the root wall of the construct and porcine aortic VICs in the leaflets of the valve [74].

5.6 3D Bioprinted Stents

3D bioprinting has been used to fabricate stents for endovascular or coronary implantation to maintain vessel patency in partially or fully occluded vessels [76]. Coronary artery disease (CAD) is attributed to plaque buildup (atherosclerosis) within the arteries affecting blood supply to cardiac muscle by narrowing the vessel lumen. Current treatments for CAD include coronary artery bypass grafting (CABG) and angioplasty [77]. CABG is usually performed as a treatment option when there is complete blockage of the vessel lumen, where an artery is grafted around the blocked areas of the artery [78]. Angioplasty or balloon angioplasty is a less invasive option for partial blockages [79]. Biomaterials for 3D printed stents often include polymers such as polylactic acid (PLA) and polycaprolactone (PCL) while stents containing metal alloys such as stainless steel and cobalt chromium are still widely used [80, 81]. For instance, fused deposition modeling (FDM) has been used to efficiently print composite polymer stents [82]. FDM is a rapid prototyping process where a thermoplastic polymer is heated to melting point and then extruded in a layer by layer fashion to create a 3D model [82]. Implantable, biodegradable, and polymer-based stents have been produced for cardiovascular applications, exhibiting minimal toxicity and suitable degradation rate for tissue remodeling [81].

5.7 Summary and Concluding Remarks

In summary, 3D bioprinting shows much promise for the future of cardiac medicine [60]. It allows complexity in engineered models, specifically allowing the cardiac construct to be personally created based on the individual patient needs. It also

allows for creating heterogeneous structures by using multiple extruders/inks for the prints, which is critical for recapitulating cardiovascular tissues with varying biochemical and physiomechanical properties (e.g., cellar composition and stiffness).

Bioprinted cardiac patches may not be fully viable yet as a clinical therapy for human patients with acute MI, until a functional vascular network is incorporated within the tissues. Co-culture integration and 3D fiber arrangement have been used to achieve some degrees of patch vascularization [34]. Other significant considerations must also be made, such as post-implantation cardiac arrhythmia occurrences among tested subjects and the patch's contractile capabilities. Implanted cardiac cells in nonhuman primates have been successfully shown to be able to remuscularize infarct primate but have been complicated by occurrences of arrhythmia [22]. These potential arrhythmic complications concomitant to cardiac patch implantation will need to be eliminated in order for patient applicability. As more advancements are made, though, with increased vascularization and little arrhythmogenicity, the plausibility of having cardiac patches available for clinical use becomes more likely.

One of the biggest setbacks with 3D bioprinting heart valves is that little testing has been done on the printed models under dynamic, physiologic conditions. Such conditions are essential for the accurate development of the extracellular matrix and tissue biomechanical testing [14, 83]. In addition, further testing is necessary to determine whether the valves can withstand the high pressures created by the ventricles during contraction, without tearing or regurgitation of blood upstream [72]. In the future, more studies will be needed to see whether these valves would be functional in an in vivo setting and if they would be a viable replacement for mechanical and biological valves.

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