



Review

Prospect of 3D bioprinting over cardiac cell therapy and conventional tissue engineering in the treatment of COVID-19 patients with myocardial injury

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ABSTRACT

Due to multiple mutations of SARS-CoV-2, the mystery of defeating the virus is still unknown. Cardiovascular complications are one of the most concerning effects of COVID-19 recently, originating from direct and indirect mechanisms. These complications are associated with long-term Cardio-vascular diseases and can induce sudden cardiac death in both infected and recovered COVID-19 patients. The purpose of this research is to do a competitive analysis between conventional techniques with the upgraded alternative 3D bioprinting to replace the damaged portion of the myocardium. Additionally, this study focuses on the potential of 3D bioprinting to be a novel alternative. Finally, current challenges and future perspective of 3D bioprinting technique is briefly discussed.

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1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which gives rise to COVID-19, is the reason behind the current global health emergency. According to WHO, 169,597,415 people are infected, including 3,503,582 deaths, as of 30th May 2021 since its origin in December 2019 [1]. Though it is considered that SARS-CoV-2 affects the respiratory system, direct and indirect involvement of the cardiovascular system has been identified in various studies [2–6], and the associated complications are closely related to worse prognosis and mortality [7]. In addition to that, medications used to treat COVID-19 are responsible for indirect cardiovascular complications and adverse side effects [8–11]. COVID-19 drugs initiate different cardiovascular problems like bundle branch block, AV block, ventricular arrhythmia, cardiomyopathy, myocardial injury, hypertension, along so many other long-term complications [12–17]. Though conventional techniques like, Cardiac cell therapy and myocardial tissue engineering are capable of producing new tissues, studies show various limitations of these techniques that make these techniques impractical to use [18,19]. In search of an appropriate and better alternative, we find 3D bioprinting one of the modern biotechnological developments which can deposit biomaterials and bioactive cells onto a computer-aided predetermined design in a layer by layer manner to print the construct [20].

Moreover, this technique can produce a highly continuous and stable biological pattern of the native heart with high-resolution stimulation that facilitates finding ways of myocardial tissue repair and regeneration [21,22]. 3D bioprinting is the technique for regenerating an utterly new tissue or organ implanted into the host. Back in 2019, the world's first patient-specific vascularized 3D bioprinted heart was tiny in size by Professor Tal Dvir and his team that paves the way for customizing organs when needed [23]. The first full-size 3D bioprinted human heart model has already been fabricated by Adam Feinberg and his team recently [24]. However, it is essential to realize that 3D bioprinting technology for heart regeneration is still in the early stage of development; a long way is needed to gain accuracy. This review provides insights into this technology and addresses all the reasons behind using this technology to treat the myocardial tissue damage causing by SARS-CoV-2 and the current challenges. Finally, the future perspectives have been discussed.

2. COVID-19 and myocardial injury

Numerous information is available, indicating the relationship between COVID-19 and damage in the cardiovascular system [25–32]. A proven case of direct cardiac damage has been published already, confirming the presence of viral particles inside the myocardium of a patient with COVID-19 and cardiogenic shock. They underwent endomyocardial biopsy [33]. 78 out of 100 individuals who recovered from COVID-19 were evaluated after 71 days of treatment, and reports reflected cardiovascular involvement despite preexisting conditions and severity of COVID-19 disease. 76% of them had high sensitivity troponin, and 60% had myocardial inflammation, edema, and diffuse myocardial fibrosis.

An epidemiological study reports that from 12% to 23% of hospitalized COVID-19 patients have increased levels of cardiac biomarkers, which can increase up to 46% in the critically ill patients and the non-survivors [25]. One of the reports of NHC about COVID-19 says, around 11.8% of patients who died from COVID-19 had substantial heart damage and increased troponin I levels or cardiac arrest [34]. A study done with 41 COVID-19 patients reports a 12% incidence of virus-related acute cardiac injury and elevated serum cardiac biomarkers in 33% of patients [25]. Few case reports

indicate the presence of severe acute myocarditis, myocardial injury, myocarditis, acute coronary syndrome, arrhythmias, heart failure, venous thromboembolism, and so many other complications. Cardiovascular comorbidities including hypertension, diabetes, preexisting cardiovascular complications are associated with adverse outcomes in COVID-19 patients [25,26,28,35]. Shi et al. reported that elevated troponin had an independent risk factor associated with death after completing the adjustment of all the other confounders. According to the evidence, around 19.7% of COVID-19 patients had a myocardial injury. The rate of hospital mortality was 51%. However, 4.5% of patients did not have raised troponin, which lowers the chance of death [7]. The first hospitalized patients at Wuhan report shows that 7.2–12% of patients were having elevated hs-cTnI, 80% of them required intensive care because of the myocardial damage [25].

Furthermore, a Chinese case series that analyzed the mortality data of 150 patients found that 5/68 patients had myocardial damage because of the viral infection, which leads to circulatory failure and thus death. 22/68 of them had myocardial damage along with respiratory failure [28]. A possible reason for COVID-19 myocardial injury can be the high expression of ACE2 on the cell surface of myocardial cells [36]. It can indicate that the virus might cause cardiac injury by direct invasion, and few case reports containing COVID-19 patients support this hypothesis [37]. Chen et al. found that the amount of ACE2 expression is higher in the pericytes of adult human hearts, which indicates that the heart is susceptible enough to be affected directly through SARS-CoV [38].

Moreover, the external catalytic effect of ACE2 is lost after binding with SARS-CoV-2 [39,40], which results in the down-regulation of ACE2 and decrement of angiotensin 1–7 level. Thus the heart function of COVID-19 patients may also be compromised [41]. Viral infections are responsible for infectious myocarditis and trigger an antiviral immune response, including virus-specific T lymphocytes, macrophages, and natural killer cells [42]. Moreover, abnormal T cell and monocyte responses are responsible for systemic hyper inflammation characterized by the higher amount of proinflammatory cytokine and chemokine production [25]. These lead to myocardial damage in COVID-19 patients. Kawasaki disease, a manifestation of macrophage activation syndrome, has also been seen in COVID-19 patients, and it supports the hypothesis of increased systemic hyper inflammation in COVID-19 disease [43]. It has been revealed that the excessive hyper-inflammation capability of SARS-CoV-2 can trigger multiple cardiovascular complications, including heart failure, stroke, tachycardia, unstable angina, and acute myocardial infarction [44]. Aberrant expression of proinflammatory cytokines TNF- α , IL-1 β , IL-6, and MMPs (Matrix Metalloproteinases) can induce myocardial infarction [45].

Myocardial injury can also happen because of the imbalance between oxygen supply and demand, classified as type 2 myocardial infarction [46]. Various studies show the presence of severe respiratory complications and hypoxia in COVID-19 patients [30]. A meta-analysis of 19 studies including 2874 patients indicates bilateral pneumonia with ground-glass opacity of 68.5%, related to hypoxia. Hypoxia also contributes to tissue inflammation which in turn causes cardiac tissue damage. Additionally, COVID-19 induced respiratory troubles can cause type 2 myocardial infarction [47]. Highly hypoxic patients with acute respiratory syndrome have a higher chance of having a worse cardiac injury, heart failure, and prognosis while in combination with systemic inflammation or cytokine storm [48]. Viruses can cause heart failure through immune and inflammatory mediated myocardial damage. In case of acute viral infection, the release of pro-inflammatory cytokines and recruitment of proinflammatory macrophages and granulocytes would be higher, leading to severe inflammatory storm and causes initial injury of the heart [49]. In combination with increased

metabolic demand, it can cause cardiac depression and new-onset Heart Failure or acute decompensation of chronic Heart Failure [50]. Microemboli or coronary microvascular dysfunction is one of the mechanisms of acute coronary injury [51], leading to heart failure [52]. An investigation found that around a quarter of hospitalized COVID-19 patients and one-third of COVID-19 patients admitted in the ICU had new onset of heart failure [26]. However, they did not have any history of heart failure [53]. Study shows that COVID-19 can cause fulminant myocarditis, which causes left ventricular dysfunction and cardiogenic shock and proves the relationship between hyper-inflammatory syndrome induced by COVID-19 and myocardial dysfunction [43]. A meta-analysis of 43 studies concerning 3600 COVID-19 patients manifests that 17.1% of patients have a prevalence of heart failure, which causes various complications to critically ill patients while recovering from the disease compared to 1.9% of non-critically ill patients [54]. Acute myocarditis can be caused by acute viral infections [55]. Critical SARS-CoV-2 infection can induce aggression to the myocardium and lead to myocarditis [56], such as segmented wall motion abnormality or reduced left ventricular ejection fraction (LVEF). Investigations in Italy [57] and China [38] indicate that in COVID-19 patients, severe myocarditis can cause low cardiac output syndrome. Mainly it is caused by a “cytokine storm” that occurs due to systemic inflammation [58]. The report claimed that cytokine storm is directly associated with cardiotoxicity [59]. An investigation of 150 COVID-19 patients observed that the non-survivors had an increased level (100%) of ferritin and IL-6 than the survivors [28]. Additionally, patients with severe disease had an increased serum of IL-6, IL-10, IL-2R, and TNF- α [60]. These cytokines can cause systemic inflammation and lead to cardiac injury. The research was done with individuals who had recovered from COVID-19 and found that 60% of them have myocardial inflammation despite preexisting cardiovascular conditions, which is a clear indication of the long-term consequence of COVID-19 [61]. SARS-CoV-2 induced myocarditis is an important acute ventricular dysfunction correlated with diffuse myocardial edema [62]. Several cases have been published already which prove the presence of myocarditis in COVID-19 patients. Both tachy and bradyarrhythmias (cardiac arrhythmias) are common in COVID-19 patients [63]. 16.7% of Chinese COVID-19 patients had arrhythmia [64] and around 44.4% need ICU admission [65]. It can occur along with myocarditis, myocardial ischemia, and critically ill patients with shock and hypoxia [66]. According to Li et al., 21% of 1051 ICU admitted COVID-19 patients developed new-onset arrhythmias. The associated risk factors can be severe sepsis, septic shock, ARDS, acute renal dysfunction, electrolyte disturbances, and patients on ventilators and vasopressors [67]. The interaction between SARS-CoV-2 and the RASS system causes electrolyte imbalance, increasing the risk of arrhythmias [68]. Electrophysiologic evidence shows that right ventricular arrhythmias can be caused by COVID-19, related to mortality [69]. Guo et al. showed the relationship between ventricular tachycardia or ventricular fibrillation with/without myocardial injury. Patients with myocardial injury had a higher rate of getting ventricular tachycardia or ventricular fibrillation, and this study suggests the interlink of arrhythmia and cardiovascular system in COVID-19 patients [31]. COVID-19 can cause both arterial and venous thrombosis [70]. COVID-19 infected individual has a higher chance of having venous thrombosis [71]. Clotting and development of coagulopathy is a deadly combination for severe and critical COVID-19 patients. A study including 184 patients showed that around 27% of patients had VTE, and 3.7% had arterial thrombotic events with the help of CT angiography and ultrasound [72]. Most of the studies use the amount of D-dimer as a reference for VTE. In the study of Zhou et al., 81% of COVID-19 patients died with elevated D-dimers (>1 g/L) [26] (Fig. 1).

The medicines used to treat COVID-19 are also be responsible for myocardial injury. For instance, to prevent the entry of SARS-CoV-2 through ACE2, ACEIs and ARBs are being used [43] like chloroquine and hydroxychloroquine. Though these medicines inhibit viral entry and decrease the chance of sudden cardiac death, they cause QTc prolongation, bundle branch block, AV block, ventricular arrhythmias [47], cardiomyopathy [10], and direct myocardial toxicity [73]. Various monoclonal IL-6 receptor inhibitors like tocilizumab and siltuximab, anti-interferon-gamma antibodies – Emapalumab, azithromycin, and corticosteroids—were examined ensure the efficiency and have proven their efficacy already [74]. Though tocilizumab inhibits IL-6 and cytokine storm, it causes a type of rare hypertension [47], hypercholesterolemia [73]. Moreover, it is responsible for decreasing hepatic LDL receptor expression and increasing serum LDL cholesterol [75], which can cause atherosclerotic cardiovascular disease.

3. Conventional treatments to treat myocardial injury

3.1. Cardiac cell therapy

Studies found that exogenous cell transplantation into damaged myocardium can improve myocardial function and vascular supply [76]. Transplanting cells to the damaged region for cardiac repair is known as cardiac cell therapy [77]. In situ cellular cardiomyoplasty, cells are being injected directly or intravenously into the infarct [78]. Various types of cells including, fetal cardiomyocytes, neonatal cardiomyocytes, autologous skeletal myoblasts, bone marrow stem cells, embryonic stem cells, adipose-derived mesenchymal stem cells, induced pluripotent stem cells, have been used for this purpose. Reports claim that the mammalian heart has intrinsic regenerative potential, and cardiac stem cells (CSCs) can differentiate into cardiomyocytes after in-vitro stimulation with oxytocin. Moreover, CSCs assist myocardial regeneration in infarcted hearts of rat models. Besides all the cell sources, a novel alternative cell source is induced pluripotent stem cells (iPSCs), which can be obtained by treating differentiated adult cells with four genes (Oct3/4, Sox2, c-Myc, and Klf4) [79] or (OCT4, SOX2, NANOG, and LIN28) [77].

3.2. Conventional tissue engineering

Tissue engineering (TE) approaches for cardiac damages have been studied recently. As myocardial tissue lacks regenerative capability, TE can be a potential alternative for restoring the functionality of damaged myocardial tissue [18]. There are three fundamental elements for a successful TE; cells, extracellular matrix, biomimetic signal, and bioreactors [80]. The primary approach is to seed cells capable of forming cardiomyocytes onto a scaffold in vitro and introducing the construct in the infarcted region [78].

4. Limitations of cardiac cell therapy and tissue engineering

Though various advancements have been introduced in the treatments of injured myocardium, the adult heart cannot regenerate effective cardiomyocytes after being injured or infarcted [81], leading to regional contractile dysfunction [18]. If the injured area is large, it can degenerate the remaining myocardium and lead to congestive heart failure [18]. Various reports show the long-lasting cardiovascular effect of COVID-19 on the heart, which indicates that the injured area is enormous and can cause sudden death to the recovered COVID-19 patients.

Collecting the correct number of cells for transplantation is a challenge. Fetal cardiomyocytes were capable of limiting scar expansion and heart failure in rat models. Thus, researchers started

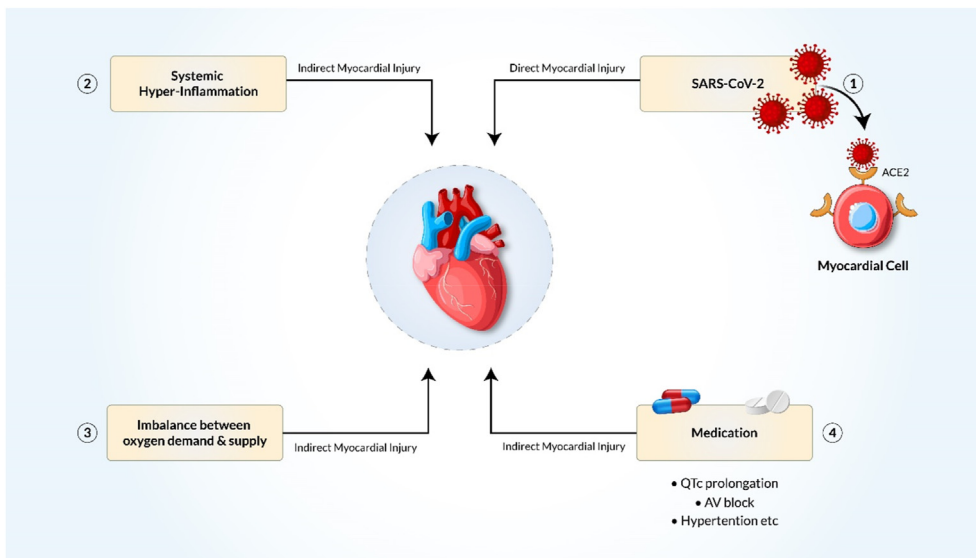


Fig. 1. Schematic diagram of mechanisms of COVID-19 induced myocardial injury. (1). The attachment of SARS-CoV-2 spike proteins causes direct myocardial injury with highly expressed ACE2 receptors of myocardial cells. (2). Introduction of the virus into myocardial cells can induce the release of inflammatory molecules like TNF- α , IL-1 β , IL-6, IL-10, virus-specific T lymphocytes, monocytes MMPs, NK cells, macrophages, etc., together cause systemic hyper-inflammation. (3). Infection in the respiratory tract induces the imbalance between oxygen demand and supply known as Type 2 Myocardial Infarction or hypoxia. Thus, indirectly damage the myocardium. (4). Medications used to treat COVID-19 are also responsible for indirect myocardial injury as they cause complications like QTc prolongation, AV block, arrhythmias, heart failure, hypertension, etc.

working with it and found out the benefits. However, using fetal cardiomyocytes in humans is not feasible as acquiring enough human fetal tissue is challenging [18,82]. Rodrigues et al. have mentioned the advantages and disadvantages of all the cells used in cardiac cell therapy. Different cells can have additional penalties. For example, cardiomyocytes (adult, fetal and neonatal) are unable to reproduce in-vivo. Thus the survival rate is shorter. Moreover, poor integration with host tissue in porcine has also been observed. Immunogenicity and risk of myocardial complications are associated with most of the cells used in the process [83].

Evidence has been collected from animal models and clinical trials that different cell sources can cause various side effects in patients after being treated with Cardiac cell therapy. The utilization of embryonic stem cells for cell therapy can increase the chance of tumorigenesis [84,85], stem cell metastasis [86], immunogenicity [87]. Skeletal myoblasts can create adverse effects like arrhythmogenesis [88,89].

Another challenge of cardiac cell therapy is determining the amount of injury in a patient. There is a difference in the benchmark for reproducible engraftment of large numbers of cells based on myocardial damage. The engraftment for early post-myocardial infarction patients is different from the patient with end-stage cardiac dysfunction [90].

Increased risk of arrhythmias even after feasible myoblast transplantation is one of the significant concerns of cardiac cell therapy [91]. If the transplantation is successful, the transplanted cells must transdifferentiate into cardiomyocytes and survive for a long time to serve the need. However, the infarcted region and the surroundings do not help the cells survive in the optimal environment [18], making the process inefficient. A significant disadvantage of tissue-engineered products is the destructive host response. After introducing these engineered constructs into the human body, they induce an innate immune response known as foreign body response along with an adaptive immune response if it contains an immunologic biological component. Chronic inflammation can also be initiated after implantation of the engineered construct. With the combination of all these features, a high chance of graft rejection initiates after the introduction [92].

Mantakaki et al. present the advantages and disadvantages of the materials and biomaterials used in tissue engineering. The study clearly shows the number of disadvantages is more than the advantages, which include the toxic effect of biomaterial scaffolds, inflammation risk, the chance of poor vascularization if more than 3-sheets are being used, the requirement of lifelong treatment with anticoagulants, and many more which indicates the ineffectiveness of the biomaterials. Additionally, they pointed that materials without having all the required characteristics can induce a non-functioning heart associated with various complications [93].

Myocardial tissue-engineered constructs use multiple defined and undefined cells which support tissue formation, vascularization, secrete signaling molecules for cardiomyocyte survival, proliferation and maturation. However, long-term survival of the myocardial grafts cannot be possible as sufficient nutrient supply is crucial in in-vivo conditions [94].

Fabrication of aligned and thick cardiovascular tissue is very hard as all the cells need to have a microvascular network for exchanging nutrients and oxygen [95]. Though several tissue engineering technologies have created cardiac patches with large surface areas, vascularization remains a significant challenge that affects oxygen supply in cells both in vitro and in vivo [96]. Researchers have tried various ways to overcome the problem already like, pulsatile flow supply around cardiomyocytes [97], development of perfusion bioreactor to ensure homogeneous fluid flow and cell exposure to perfusion medium [98,99], minimization of intrinsic diffusional constraints [100], utilization of in-vivo mechanisms of convective-diffusive oxygen transport [101–105]. Individually, these techniques cannot elevate vascularization for a long time. The exact feature of vascularization that needs to be modified is unclear, making the procedure even more complicated [96]. According to Wang et al., other limitations of tissue engineering are the inconsistency of stiffness between the engineered tissue construct and the myocardium, which can be responsible for implanting a dysfunctional construct with poor engraftment inside the human body [77].

Engineering large cardiac constructs through tissue engineering are challenging as cardiac tissues are composed of ECM proteins and

different cells with different alignments to make spontaneous contraction possible. All the properties of the native myocardium cannot be maintained throughout. As a result, the construct cannot contract like the native one, and the cells died after a few times [95].

Though different organs can be made by 3D scaffold-based tissue engineering, successful results are only observed for avascular organs. Developing a highly vascularized organ like the heart is challenging to fabricate to be functional for a more extended period because of the massive number of branched blood arteries and capillaries [95]. Moreover, seeding cells on these scaffolds lead to cell death and can cause poor cellular performance [106].

5. 3D bioprinting to treat myocardial injury

3D bioprinting is a bio-fabrication method as it can deposit various cells/tissues onto a pre-decided location based on a computer-aided design (CAD). 3D bioprinting can control the structure of the cell-biomaterial architecture and provides the required physicochemical and biological environment for the maintenance and maturation of the tissue construct. Various types of bioprinting techniques are already available, and those can be categorized into four different modules depending on the working principle: (1) droplet-based, (2) extrusion-based, (3) laser-assisted, and (4) stereolithography techniques. A perfect bio-ink should be selected for the fabrication to make a 3D structure that mimics the actual organ/tissue. To achieve the ideal bio-ink, the mixture of different biomaterials based on the mechanical properties acquire attention [106]. The perfect 3D structure, along with required functions, can be achieved with the help of printable biomaterials (bioinks), 3D bioprinters of different techniques, and microenvironmental regulations for promoting tissue morphogenesis.

The whole Bioprinting procedure can be divided into three distinct stages. The first one is the pre-processing stage, where all the planning for successful bioprinting is present. Imaging is a must to analyze the overall structure, which will help make the blueprint for bioprinting. The second phase is Bioprinting. All the complexity is present in this stage. Choosing the right bioprinting technology and the appropriate bioink and cell is tricky as all these things can manipulate the effectiveness of the printed 3D construct. Moreover, the bio-ink preparation is a bit technical as it requires a suitable cell source, the perfect scaffold materials, and the additives like growth factors, chemicals, microcarriers, etc. [74]. Cell viability is one of the most influencing factors that can help to get a preferable result out of the 3D bioprinted construct. Cell viability depends on factors such as the duration of the whole printing procedure, the sensitivity of the cells, etc. Selecting the appropriate bioprinting technique that requires less time to complete the whole procedure along with supplying all the required components to the cells (culture media) will be helpful enough to secure the required number of cells [112]. Finally, the third stage is the post-bioprinting stage, and it includes all the steps that need to be done to get a mature and fully functional bioprinted construct for in-vivo usage [74]. To evaluate the quality of the bio-printed construct various in vitro analyses can be done. Assessment of cardiac biomarkers, efficient contractile force (ECF), spontaneous action potential (SAP), and overall calcium regulation (OCR) will be of great help. Estimation of the muscle construction, depolarization-repolarization action potentials of the newly bioprinted construct will determine the quality. Additionally, transplantation of the construct into the infected hearts of immune-deficient rats can deduce the orthotopic safety and effectiveness of the 3D bioprinted construct [20]. The transplanted construct helped reduce the infarction area, increased neovascularization, and enhanced the ejection fraction and cardiac output, thus showing a remarkable therapeutic effect [20]. Several in vivo

studies have already evaluated the performance of the 3D bioprinted construct to treat myocardial infarction. 3D bioprinted prevascularized and functional constructs stimulated strong vascularization, tissue matrix formation after implanting into rat hearts which is a positive indication for utilizing personalized 3D bioprinted construct to humans [120]. To treat COVID-19 patients with 3D bioprinted construct considerations such as the presence of comorbidities, lack of nutrient supply to the cells, presence of inflammatory cytokines in the surrounding tissues must be taken care of as these factors can lead to sudden cardiac death. Determining the amount of all these factors can be beneficial to decide to utilize 3D bioprinting for the patient [7] (Fig. 2).

6. Why 3D bioprinting is a better alternative for treating COVID-19 myocardial injury

Factors such as, age, preexisting cardiovascular diseases, cerebrovascular diseases, diabetes mellitus, chronic obstructive pulmonary disease, ARDS, higher creatinine level of COVID-19 patients increase the risk of greater cardiovascular damage that eventually lead to death [7]. The ratio of myocardial damage can determine whether the heart will regain its competence or not. A highly injured myocardium can also cause sudden cardiac death or long-lasting cardiovascular complications [18]. Tissue engineering of the larger damaged area of the myocardium is difficult because of its complex nature [109]. 3D bioprinted heart will be the most efficient solution to prevent long-lasting cardiovascular complications or sudden cardiac death, as it is capable of mimicking the complex structure of the myocardium [107]. To ascertain the therapeutic potential of the 3D bioprinted construct, 3D bioprinted constructs have been transplanted in mouse MI model, and the outcomes have been analyzed. Surprisingly, a cardiac function has been improved with the help of the transplanted cells as they influenced tissue remodeling. Besides vascularization and prolonged cell survival have been evident that indicates the capillary network formation for nutrient and oxygen supply into the cells [110].

Compared to conventional tissue engineering techniques, 3D bioprinting can influence the stem cells to differentiate during various stages of the whole procedure. This influence of differentiation can vary based on the choice of stem cells, bioprinting method, selection of scaffold, additives, and mechanical forces. Moreover, these stem cells have immunotolerance and can expand after being incorporated into the target tissue. To promote differentiation, even more, microcarriers can be a source of great help. These small polymer spheres can influence the differentiation after being added to the bioink and can solidify the scaffold. This differentiation influence is significant for the overall potential of the 3D bioprinting process, which makes the technique better than the rest [74].

The 3D bioprinting process utilizes two types of photo cross-linkable hydrogels: rigid works for the root, and soft, which works for the leaflets. The printing procedure was extrusion-based, and within 45 min, the heart was printed, indicating that 3D bioprinting is a speedy technique compared to any other method for myocardial regeneration [108].

Generally, the other regeneration techniques emphasize more on the shaping capability of the ink. On the other hand, the 3D bioprinting technique focuses on producing high biocompatibility and biodegradability of the bio link to help merge the living cells or bioactive molecules. The use of hydrogel dECM has excellent potential in 3D CTE bioink preparation. Cardiac dECM is capable of reproducing the same physical and mechanical microenvironment along with therapeutic potential. Proteomic research of the physiological and pathological dECM of the cardiological system will be helpful to extricate more functional factors which support the production of advanced 3D CTE bioink [20].

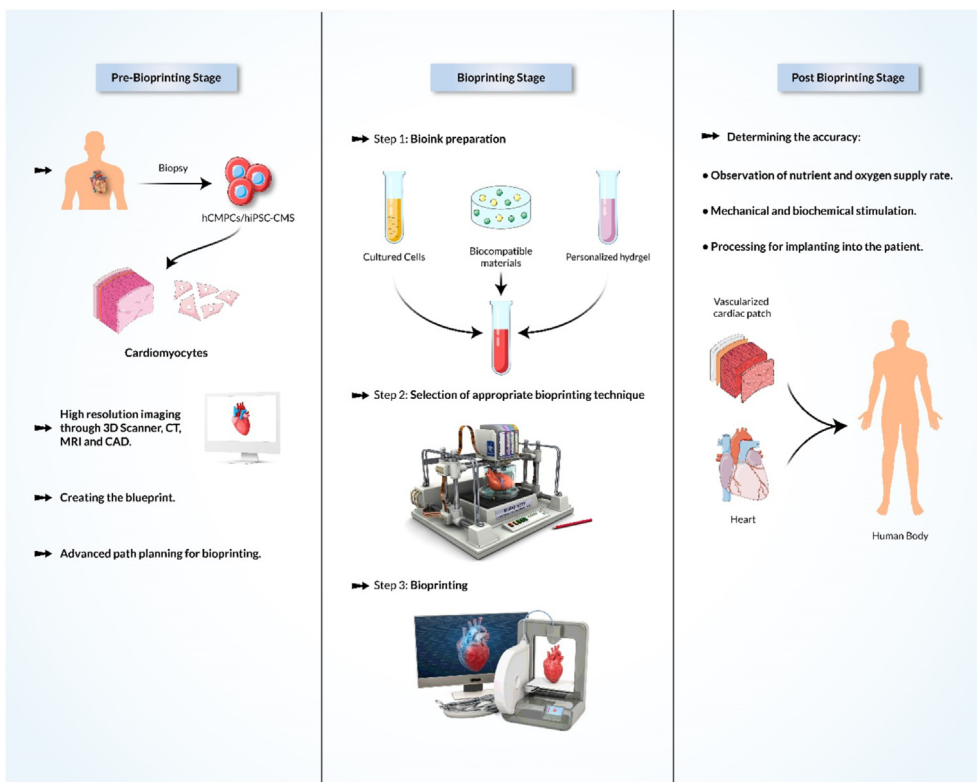


Fig. 2. 3D bioprinting of the heart. The pre bioprinting stage starts with tissue biopsy for collecting samples, then the cells are differentiated into cardiomyocytes. Next, high-resolution imaging is done through a 3D scanner/CT/MRI or CAD can be done for creating the blueprint of the native myocardium. This stage ends with advanced planning for bioprinting. The bioprinting stage has three consecutive steps. Firstly, the bio-ink is being made with the culture cells, personalized hydrogel, and bio-molecules. Secondly, the appropriate bioprinting technique is selected, and then the bioprinting proceed. The post bioprinting stage determines the accuracy of the printed construct for effective implantation. Nutrient and oxygen supply rates are determined along with mechanical and biochemical stimulation. Finally, transplanted into the patients.

Another advancement of 3D bioprinting that makes this technique a better alternative is the high-fidelity replicating of supple and tough textures de novo. For example, the printing of many functional skeletal muscles. This has been possible by the precise control of the composition, structure, and shape before producing the construct [20].

3D bioprinting can develop heterogeneous 3D scaffolds of muscular mechanical strength that pose all the required characteristics, morphology, and accuracy of the native myocardium. All of these are possible with the help of a computer-aided design (AutoCAD) facility to make a biomimetic 3D scaffold of the native heart shape [109]. Moreover, this process has precise control over various compositions, structural complexity, distribution, effective printing of all the tissues with accurate features [110].

3D bioprinting is a promising approach to generate porous inner structure, which helps to provide the required nutrients and oxygen to other tissues [111]. As the technique is automated, mass production of cells is more accessible, and the construct will be of high resolution [106]. Additionally, the 3D products contain precise architecture which is reproducible and repeatable [112].

Vascularization in the 3D printed myocardial cell construct is another outstanding advancement. Carbohydrate filaments were utilized to construct a 3D printed vascular network that can be perfused with pulsatile and high-pressure flows [113]. In another research, micropores have been used to ensure perfusion, and thereby all the metabolic processes have been maintained in 3D engineered hepatocyte tissue [114]. As a noninvasive spatial patterning technique, ultrasound standing wavefields are employed, directing endothelial cells to form vascular networks in the engineered tissues by controlling sound in the sound field [115–118]. To stimulate muscle contraction, electrical conditioning

plays a vital role by improving the conductive properties of cardiac cells and inducing contraction rate characteristics of pacemaker cells. Therefore, electrical stimulation assisted in organizing atrial and ventricular structure along with the concomitant beating of all cardiomyocytes [120].

This technique uses tissue-specific models for bioprinting the organ or specific tissues, which are helpful to test therapeutic schemes and aid in the clinical diagnosis and treatment of disease through the replacement of the injured tissues. Selection of appropriate drugs regenerative medicine can be created with a lesser amount than conventional tissue engineering. Personalized pathophysiological conditions can be determined beforehand from the genome, proteins, and medical/family history, which will help reduce life-threatening effects [111].

As the 3D bioprinting technique can regenerate customized and complex 3D models of human tissues and organs, it is beneficial to reduce animal testing. It can overcome all the ethical concerns related to animal testing [20].

Scaffolds of the engineered construct can create problems like immune reaction and degradability, which can be life-threatening. That is why the scaffold-free 3D bioprinting technique has emerged as an alternative. Here the cells are being spread in a 3D environment, and the rapid differentiation creates a solid mass by cell–cell adhesion. After the complete regeneration, the cells are being placed layer by layer with 3D printer support and develop a tissue-like structure robotically [109].

7. Current challenges and future perspective

Despite numerous advancements in 3D bioprinting, this field is not in its highest developed form yet [119]. Cardiovascular

constructs like the vascular constructs, myocardium, heart valves, tissues, etc., have already been printed successfully with distinct structures and functions [110]. However, to make 3D bioprinting successful, one challenge is selecting and preparing the perfect bioink. The bioink must pose all the essential characteristics, and it must be maintained in-vivo for a more extended period. Research is going on to develop a bioink of balanced bio printability and bio-functionality [106]. As the bioprinting technique needs bioactive polymers for the procedure, the selected molecules are sometimes biologically so active that they can cause unwanted cellular interactions and unfavorable stem cell differentiation. Thus, choosing the appropriate biomolecule can be a challenge [74]. Another challenge can be the selection of fabrication strategy as the complex organs contain cells of different patterns. Thus, the printing may require various printing techniques to introduce structural heterogeneity and functionality [112]. Finally, for effective industrial translation and commercialization of 3D printed organs, quality assurance and regulation of bioinks, bioprinters, and bioprinted products are a must. Maintaining the quality in all the steps of 3D construct regeneration can be a little more complicated. On May 10, 2016, FDA issued draft guidance regarding “Technical Considerations for Additive Manufactured Devices” that offer a clear idea to the manufacturers and all of this guidance’s must be followed to get the approval from FDA. Ethical concerns can also cause problems while implanting the 3D bioprinted construct in humans [112].

Recent advancements in the 3D bioprinting discipline indicate the tremendous potential of this technique in developing functional tissues and organs for clinical transplantation [107] and developing the cardiac tissue engineering field in the innovation of relevant treatment procedures for cardiovascular diseases [120]. Analyzing the aspects of 3D bioprinting technology in cardiology more will be beneficial in solving the existing problems through practical updates [20]. Combining the high-throughput 3D bioprinting technology with stem cell technology will be a powerful concept in tissue engineering and regenerative medicine [20]. However, to generate a fully functional organ, 1–10 billion function cells are required, and the current technology is still unable to grasp the ultimate target. Thus, improving and maintaining cell density in tissue construct is still a challenge in 3D bioprinting that needs proper attention [121]. Various technologies are being integrated with 3D bioprinting technology, shifting today’s 3D bioprinting technology paradigm. Integration of electronics, biosensors, and engineered tissue devices will be beneficial to create functional living cells like cardiac muscles with electrophysical signals. It will provide new tissue morphogenesis, pathogenesis, drug-responsive remodeling processes, and personalized medicine establishment [111]. Continuous chain study is needed to gain such technological advancement along with corresponding epigenetic mechanisms related to producing new cardiovascular constructs to make 3D bioprinting technology one of the most reliable, efficient, favorable methods to fabricate tissue constructs near the near future.

8. Conclusion

The cases of COVID-19 infected individuals and death from this disease are increasing at an alarming rate while numerous complications are being added to the list. Myocardial injury is one of the most concerning ones now as the patients and the recovered ones face sudden heart complications, leading to death. Though conventional techniques like cardiac cell therapy and tissue engineering can replace the damaged cardiac tissues, these techniques have several drawbacks and cause various side effects, leading to death. To solve the upcoming problem, 3D bioprinting shows enormous

potential as it will be helpful to generate functional, biocompatible tissue. If the damage rate is higher, it can print the whole organ and replace it with the damaged one. Studies will make this technology more appropriate to generate a heart that mimics the native nature and its features and solves the upcoming problem with accuracy.

9. Executive summary

9.1. Prospect of 3D bioprinted heart

- 3D bioprinting is one of the modern biotechnological developments utilized to print organs like the heart in a layer-by-layer manner on a computer-aided predetermined design.
- 3D bioprinting can print a heart with continuous and stable biological characteristics that mimic the native heart.

9.2. COVID-19 and myocardial injury

- Numerous case reports reveal various myocardial complications like myocardial inflammation, severe acute myocarditis, myocarditis, acute coronary syndrome, arrhythmias, heart failure, venous thromboembolism.
- Both direct and indirect myocardial injury can happen in COVID-19 infected patients.
- Attachment of SARS-CoV-2 spike proteins with highly expressed ACE2 receptors of myocardial cells causes direct myocardial injury.
- Indirect myocardial injury can be induced through systemic hyper-inflammation, oxygen imbalance, along with the medications used to treat COVID-19.

9.3. Limitation of conventional treatments to treat myocardial injury

- Cardiac cell therapy and myocardial tissue engineering are conventional treatments for myocardial injury. However, the adult heart cannot regenerate effective cardiomyocytes after being injured or infarcted. If the injured area is large, then it can degenerate the remaining myocardium.
- The limitations of conventional treatments that make these techniques inefficient are the collection of the required amount of cells, risk of tumorigenesis, stem cell metastasis, immunogenicity, arrhythmogenesis, immune rejection, etc.

9.4. Supremacy of 3D bioprinting technology

- 3D bioprinting can influence stem cell differentiation, develop heterogeneous 3D scaffolds, generate porous inner structure, high fidelity replication of supple and tough textures de novo.
- 3D bioprinting utilizes tissue-specific models, two types of photo cross-linkable hydrogels.
- Vascularization in the 3D printed myocardial cell construct is achieved through perfusion by carbohydrate filaments, micro-pore formation, employment of ultrasound standing wavefields.

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Declaration of competing interest

The authors declare no conflict of interest regarding the publication of the manuscript.

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