Prospects of Heart tissue Bioprinting for COVID-19 recovered patients with irreversible heart damage.

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A thesis submitted to the department of Mathematics and Natural Sciences in partial fulfillment of the requirement for the degree of Bachelors of Science in Biotechnology

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Declaration

It is hereby declared that

1. The thesis submitted is my original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
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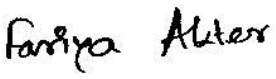

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Approval

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Ethics Statement

This thesis has been composed solely by me and it has not been submitted, in whole or in part, in any previous institution for a degree or diploma. All explanations that have been adopted literally or analogously are marked as such.

Abstract

Endomyocardial biopsy of COVID-19 patients myocardium proves the presence of viral particles along with the cardiogenic shock. 78 out of 100 recovered individuals who take 71 days of treatment had cardiovascular involvement despite recovering from COVID-19. 76% of them had high sensitivity troponin which is an indicator of myocardial damage and 60% had myocardial inflammation, edema, and/or diffuse myocardial fibrosis. Besides, the recently recovered patient's report reflects ventricular ejection fraction.

Due to multiple mutations of the causative agent SARS-CoV-2, it's still a mystery how we can defeat the virus. Numerous complications are associated with COVID-19 and studies found the association of cardiovascular complications in every individual despite the age consideration, recently. Cardiovascular complications are responsible for sudden cardiac death of both the infected and recovered COVID-19 patients along with long-term cardiovascular effects as damaged myocardial tissues are unable to regenerate and few portions of damaged myocardium can induce damage to the whole heart.

The purpose of this research is to do a competitive analysis of the conventional tissue engineering techniques with the upgraded alternative 3D bioprinting to replace the damaged portion of the human heart due to COVID-19. Additionally, this study focuses on the key points for the regeneration of a functional, biocompatible heart that mimics the native heart and the potential of 3D bioprinting to be a novel alternative. Finally, the limitations and current challenges of these techniques are briefly discussed.

Keywords: COVID-19, irreversible heart tissue damage, regenerative medicine, 3D Bioprinting.

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Chapter 1

1.1 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, 126,888,424 people are being infected including 2,778,603 deaths with this virus as of 29Th March 2021 since the origin in December 2019 [1]. Though it is being considered that SARC-CoV2 affects the respiratory system, induction of cardiac injury cannot be neglected [2], [3] as it is closely related to worse prognosis and mortality [4] despite any age consideration. Moreover, the ratio of myocardial injury starts to increase in the ICU patients which is a clear indication of a worse prognosis. Current therapeutics are also responsible for cardiovascular damage. COVID-19 drugs initiate different cardiovascular problems like bundle branch block, AV block, ventricular arrhythmia, cardiomyopathy, myocardial injury, hypertension along so many other complications. These

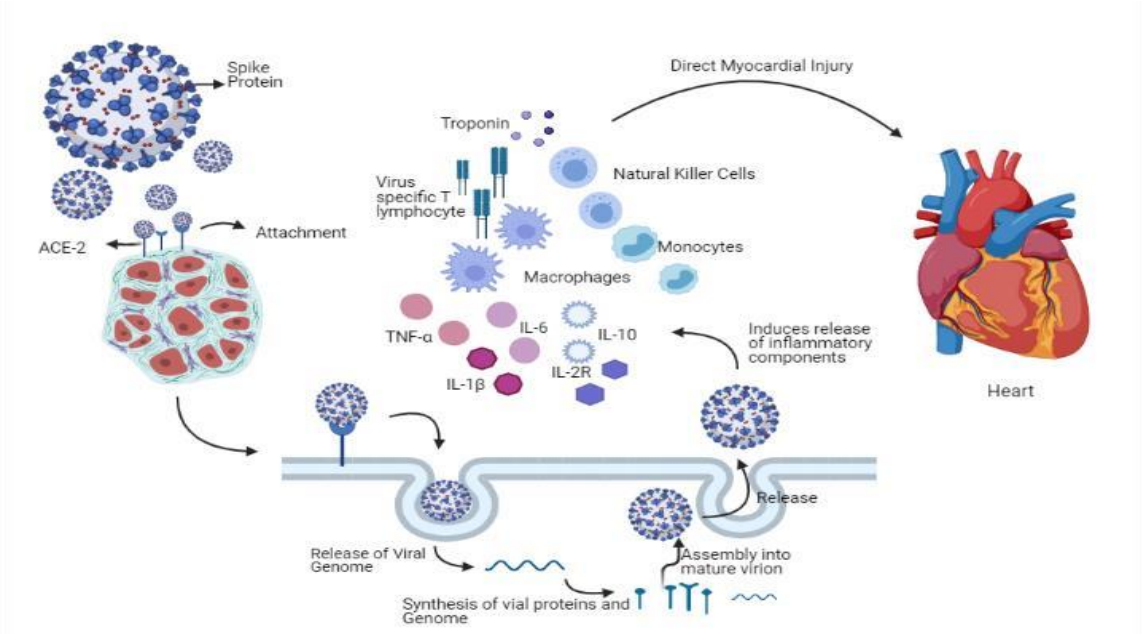

Figure 1: Entry mechanism of SARS-CoV-2 into heart

complications are correlated to cardiovascular tissue damage and have long-term effects in the COVID-19 infected individuals. Thus, a potential therapy needs to be identified that is capable of

terminating the long-term effects by replacing the injured tissue with a completely new tissue or organ. Cardiac cell therapy and myocardial tissue engineering are capable of producing new tissues and these tissues can be implanted into the infarcted region to resolve the problem. However, various questions need to be answered before implantation, whether the tissues that are produced by these techniques can survive for a longer period, whether they are biocompatible, whether they are non-immunogenic or not, and so on. Studies show various limitations of these techniques that make these techniques impractical to use. In search of an appropriate and better alternative, we find 3D bioprinting. 3D bioprinting is the technique for regenerating a completely new tissue or organ that can be implanted into the host. In this review, first of all, we provide evidence of heart complications associated with COVID-19 and medicines used to treat it. Secondly, the current technologies for heart tissue regeneration and their limitations. Finally, we provide insights into 3D bioprinting technology, address all the reasons behind using this technology to treat the myocardial tissue damage causing by SARS-CoV-2, along with the areas where this technology can be utilized. Finally, the current limitations/challenges are discussed.

1.2 Background

Studies prove the deadly myocardial effect of SARS-CoV-2 in COVID-19 patients. It is proposed that the increasing amount of cardiac biomarkers in hospitalized COVID-19 patients might have related to poor prognosis [5][3]. Moreover, SARS-CoV-2 viral particles have already been found in cardiac macrophages which is a clear indicator of virus-induced cell infection [6]**.** Reports present the sudden cardiac complications in patients after recovering from COVID-19. This indicates the adverse effect of this disease. This effect is getting accelerated with the current medications used to treat COVID-19. The rate of myocardial injury in COVID-19 patients is increasing at an alarming rate and it will be a threat to the whole world. In search of the solution, we come across cell therapy and conventional tissue engineering which are successful in reducing cardiovascular complications. However, these techniques have many limitations to fully manage cardiovascular complications by regenerating functional constructs to replace the damaged part

because of the complex composition of the myocardium and micro-vascularization. As a better alternative, we detect 3D bioprinting technology that has the potential to generate a fully functional heart construct that mimics the native myocardium, and the chance of immunogenic reaction is the least. This new field is the future for treating cardiovascular complications as studies are going to overcome the challenges. The cardiovascular complications associated with COVID-19 can suddenly rise and create another pandemic. Thus, choosing an appropriate and up-to-date solution is needed. 3D bioprinting is such a technique that is capable of reducing another chance of pandemic by replacing the injured tissues or the whole organ with a new one.

Chapter 2

2. Complications of Heart Associated with COVID-19

Numerous information is available already which indicates the relationship between COVID-19 and damage in the cardiovascular system. Patients of any age are experiencing heart complications where the patients with pre-existing co-morbidities impuissant to COVID-19 and the complications associated with it along with poor prognosis [7]. However, the second wave of COVID-19 is affecting every individual including children and young adults [8]. A proven case of direct cardiac damage has been published already which proves the presence of viral particles inside

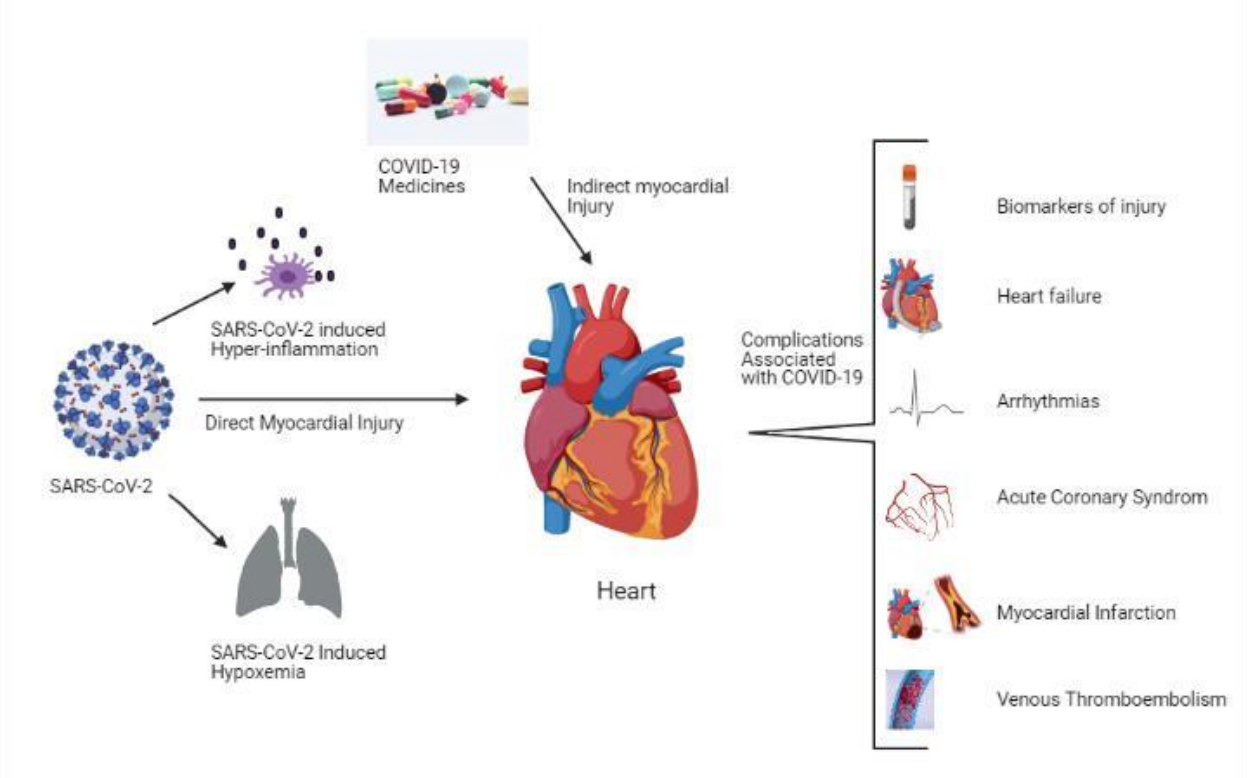

Figure 2: SARS-CoV-2 induced heart complications

the myocardium of a patient with COVID-19 and cardiogenic shock who underwent endomyocardial biopsy [6]. 78 out of 100 individuals recovered from COVID-19 were evaluated after 71 days of treatment and reports reflected cardiovascular involvement despite pre-existing conditions and severity of COVID-19 disease. 76% of them had high sensitivity troponin and 60% had myocardial inflammation, edema, and/or diffuse myocardial fibrosis. Moreover, recently recovered patients had lower left ventricular and right ventricular ejection fractions, higher left ventricular volumes and masses while compared with both healthy and risk factor matched controls [9]. Epidemiological studies have reported that from 12% to 23% of hospitalized COVID-19 patients have increased levels of cardiac biomarkers which can increase up to 46% in the patients who are critically ill and the non-survivors [10]–[14]. Moreover, this kind of data indicates the presence of serum creatine kinase (CK) and lactate dehydrogenase (LDH) in COVID-19 patients [15] and specifies tissue damage. Thus, cardiac disease is associated with COVID-19 [16]. A study shows that patients without pre-existing cardiovascular disease with elevated serum troponin levels having a mortality rate of 37.50%. On the other hand, the patients with pre-existing cardiovascular disease have a normal troponin level with a lower mortality rate of 13.33% [17]. One of the reports of NHC about COVID-19 says, around 11.8% of patients who died from COVID-19 had substantial heart damage along with increased troponin I levels or cardiac arrest [16]. A study done with 41 COVID-19 patients reports a 12% incidence of virus-related acute cardiac injury and elevated level of serum cardiac biomarker in 33% of patients [10]. A study done with COVID-19 patients who were in the hospital says that 7-17% of patients were having cardiac injury [10]. However, this kind of injury is more common among the COVID-19 patients who were in the ICU (22.2% vs 2%) and the deceased (59% vs 1%) [12], [17]. All these data prove that cardiovascular damage has become one of the most potential outcomes of COVID-19. The history of viral epidemics proves the presence of myocarditis, cardiomyopathy, HF, MI, arrhythmias, and sudden cardiac death [18], [19]. It was expected that COVID-19 would be associated with these kinds of complications. Few case reports indicate the presence of severe acute myocarditis [14],[15], myocardial injury [4][5], acute coronary syndrome, arrhythmias, heart failure, venous thromboembolism, and so many other complications. To understand how the cardiovascular system is getting affected with COVID-19, we will be analyzing all the possible mechanisms below:

2.1 Myocardial Injury

Myocardial injury can be identified by evaluating cardiac biomarkers [10]. The fourth universal definition of myocardial infarction, given by the European Society of Cardiology (ESC), the increased level of cardiac troponin (>99 percentile of reference limit) is the marker of myocardial injury [22]. Most of the reports use this reference while demonstrating the result [4], [5], [12]. Shi et al reported that after completing the adjustment of all the other confounders, elevated troponin had an independent risk factor associated with death. According to the evidence, around 19.7% of COVID-19 patients were having a myocardial injury. The rate of hospital mortality was 51%.

However, 4.5% of patients did not have raised troponin which in turn lowers the chance of death [4] The report of the first hospitalized patients at Wuhan shows that 7.2-12% of patients were having elevated hs-cTnI, 80% of them required intensive care because of the myocardial damage [10], [13]. Another report of 191 COVID-19 hospitalized patients shows a significant number of deceased COVID-19 patients had a higher rate of myocardial injury. In another case study of 419 COVID-19 patients of Shenzhen, China, two groups of patients were observed where the level of hs-cTnI was significantly higher in 36 patients who were in the ICU. After doing an echocardiogram of these patients, it was found that 11 patients had thickened interventricular septum along with enlarged left ventricular diastolic diameter. In addition to that, 4 patients had increased pulmonary arterial pressure and decreased left ventricular ejection fraction[24]. 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. Ruan et al found that the level of cardiac troponin and myoglobin were very high in the deceased group[25]. Moreover, a meta-analysis of 4 different studies proves the presence of higher cardiac troponin I level in the patients with severe complications among 341 COVID-19 patients [26]. To understand the correlation between troponin levels with COVID-19 progression, Deng et al performed a study and found that 37.5% of patients had normal troponin levels during admission which had increased while they were in the hospital and picked in the week before demise [27]. Though all the case reports talk about myocardial injury, the exact mechanism of it is still not present. However, some possible mechanisms can be responsible for inducing myocardial injury. Those are given below:

2.1.1 The role of ACE2 in Cardiac damage caused by COVID-19:

ACE2 plays a vital role in converting vasoconstrictor angiotensin II to vasodilator angiotensin 17 which helps to turn on anti-arrhythmogenic and anti-remodeling protective effects in the cardiovascular system [28], [29] and antiproliferative effects on vascular smooth muscle cells [30] and cardiac fibroblasts [31] thus, brings a beneficial effect in several pathological conditions, like CVD, diabetes, and hypertension [32]. ACE2 receptors are highly expressed on the cell surface of myocardial cells [33]. It can be an indication that the virus might cause cardiac injury by direct invasion and few case reports which contain COVID-19 patients support this hypothesis [20], [34]. 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 SARS-CoV infection [35]. However, the decrement of ACE2 concentration can also be responsible for cardiac damage as it plays a key role in cardiovascular and respiratory homeostasis [36]. Thus, transplantation of ACE2 mesenchymal stem cells can be beneficial for improving symptoms and pulmonary function of COVID-19 patients [37].

2.1.2 Imbalance between myocardial oxygen supply and demand

Myocardial injury can also happen because of the imbalance between oxygen supply and demand which is being classified as type 2 myocardial infarction [22]. Various studies show the presence of severe respiratory complications and hypoxia in COVID-19 patients [11]. A meta-analysis of 19 studies including 2874 patients indicates bilateral pneumonia with ground-glass opacity of 68.5% which is related to hypoxia. Hypoxia also contributes to tissue inflammation which in turn causes cardiac tissue damage [39]. Additionally, COVID-19 induced respiratory troubles can cause type 2 myocardial infarction [40]. Highly hypoxic patients with acute respiratory syndrome have a higher chance of having a worse cardiac injury, heart failure, and the prognosis while in combination with systemic inflammation or cytokine storm [41]

2.1.3 Systemic Hyperinflammation:

Viral infections are responsible for infectious myocarditis and trigger the activation of antiviral immune response including virus-specific T lymphocytes, macrophages, and natural killer cells [42]. Moreover, abnormal T cell and monocyte response are responsible for systemic hyperinflammation characterized by the higher amount of proinflammatory cytokine and chemokine production [10], [12] and these lead to myocardial damage in COVID-19 patients [34]. Kawasaki disease which is a manifestation of macrophage activation syndrome [43] has also be seen in COVID-19 patients and it supports the hypothesis of increase systemic hyper-inflammation happens in COVID-19 disease [44]. It has been revealed that the excessive hyper-inflammation capability of SARS-CoV-2 can trigger multiple cardiovascular complications [45] including heart failure, stroke, tachycardia, unstable angina, and acute myocardial infarction [46]. Aberrant expression of proinflammatory cytokines TNF-α, IL-1β, IL-6, and MMPSs (Matrix Metalloproteinases) can induce myocardial infarction [47]. Multiple studies proclaimed the relationship between systemic hyper inflammation and heart palpitations along with tachycardia in COVID-19 patients [48].

2.1.4 Heart failure (HF):

Viruses can cause heart failure through immune and inflammatory mediated myocardial damage [49]. Acute systematic inflammation and septic shock can be responsible for myocardial damage by increasing left ventricular end-diastolic volume along with myocardial depression [44]. Additionally, enterovirus-mediated myocarditis can be caused by excessive T lymphocyte response which can provoke left ventricular dilation and/or dysfunction [50]. Finally, a high level of circulating cytokines induces deterioration of myocardial cell contraction and relaxation in vitro [51]. In case of acute viral infection, the release of pro-inflammatory cytokines and recruitment of proinflammatory macrophages along with granulocytes would be higher which can lead to severe inflammatory storm and causes initial injury of the heart [52]. In combination with increased

metabolic demand, it can cause cardiac depression along with new-onset Heart Failure or acute decompensation of chronic Heart Failure [53]. 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 [12], [54] though they did not have any history of heart failure [55] Study shows that COVID-19 can cause fulminant myocarditis which in turn causes left ventricular dysfunction and/or cardiogenic shock [61], and proves the relationship between hyperinflammatory syndrome induced by COVID-19 and myocardial dysfunction [44]. A meta-analysis of 43 studies concerning 3,600 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 [58].

2.1.5 Myocarditis:

Acute myocarditis can be caused by acute viral infections [59]. Critical SARC-CoV-2 infection can induce aggression to the myocardium and lead to myocarditis [60] such as segmented wall motion abnormality or reduced left ventricular ejection fraction (LVEF). Investigations are done in Italy [34] and China [20] indicate that in COVID-19 patients, severe myocarditis can cause low cardiac output syndrome. Mainly it is caused by a "cytokine storm" that occurs as a result of systemic inflammation [61]. The report claimed that cytokine storm is directly associated with cardiotoxicity [62]. An investigation consisting of 150 COVID-19 patients observed that the nonsurvivors had an increased level (100%) of ferritin and IL-6 compared to the survivors [25], [63].

Additionally, patients with severe disease had an increased level of serum IL-6, IL-10, IL-2R, and TNF-α [64]. These cytokines can cause systemic inflammation and lead to cardiac injury [65]. The research was done with individuals who had recovered from COVID-19 and found that despite having any preexisting cardiovascular conditions, 60% of them are having myocardial inflammation which is a clear indication of the long-term consequence of COVID-19 [9]. SARSCoV-2 induced myocarditis is an important acute ventricular dysfunction correlated with diffuse myocardial edema [66]. Several cases have been published already which prove the

presence of myocarditis in COVID-19 patients. A 53-year-old woman was having fatigue and hypotension with diffuse ST elevation and elevated troponins were present thus, coronary angiography was done. However, the result was negative. Considering the current outbreak, the medical team thought of myocarditis and their hypothesis proved right when the patient was found to be COVID19 positive [21]. A report by Fried et al. discussed a patient with chest pressure who was eventually found to be COVID-19 positive along with myocarditis [67]. To understand the long-term survival rate, 112 recovered COVID-19 patients were chosen with biopsy-proven myocarditis. A significant number of patients died during follow up and other series of patients with myocarditis indicated reduce survival rate and sudden cardiac death [68].

2.2 Arrhythmias:

Both techy and brady-arrhythmias (cardiac arrhythmias) are common in COVID-19 patients [7].

16.7% of Chinese COVID-19 patients had arrhythmia [11] and around 44.4% of them need ICU admission [69]. It can occur along with myocarditis, myocardial ischemia, and in critically ill patients with shock and hypoxia [70]. According to the report of Li et al, 21% of 1051 ICU admitted COVID-19 patients developed new-onset arrhythmias and the associated risk factors can be severe sepsis, septic shock, ARDS, acute renal dysfunction, electrolyte disturbances, and patients on ventilator and vasopressors [71]. Electrophysiologic evidence shows that right ventricular arrhythmias can be caused by COVID-19 which is related to mortality [72]. 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 [5].

2.3 Venous thromboembolism (VTE):

COVID-19 can cause both arterial and venous thrombosis [73]. COVID-19 infected induvial has a higher chance of having venous thrombosis [74]. Clotting and development of coagulopathy is a deadly combination for severe and critical COVID-19 patients [75], [76]. 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/or ultrasound [77]. Most of the studies use the amount of Ddimer as a reference for VTE. In the study of Zhou et al, 81% of COVID-19 patients died with elevated D-dimers (>1g/L) [12]. Another study found that elevated D-dimer was associated with severe outcomes in patients [75]. Deep venous thrombosis was identified in COVID-19 patients with the help of ultrasound among those, 27% were in the ICU [77].

2.4 Medication of COVID-19 and related myocardial complications

To mitigate the immediate problem and for saving lives, various types of medicines are already being used. Though these medicines are helpful to mitigate current problems, studies found that these medicines are associated with long-term heart complications. In this part, different medicines of COVID-19, their uses, and the side effects will be discussed.

ACE2 is widely expressed in cardiac pericytes, cardiomyocytes [35], so SARS-CoV-2 can easily enter into cardiomyocytes and cause myocardial damage. To prevent this, ACEIs and ARBs are being used [44]. A multicenter study containing 1,128 hospitalized hypertensive COVID-19 patients had been given ACEIs (Angiotensin-converting enzyme Inhibitors) or ARBs (Angiotensin II receptor blockers) and lower mortality risk compared to the nonuser hospitalized patients [78]. European Society of Cardiology and The International Society of Hypertension have already permitted the use of these, though multiple papers suggested that these inhibitors will have adverse effects [7].

Systematic inflammation has the potential to affect the heart [65] and can cause problems like myocarditis and heart failure. To minimize this toxic effect different inhibitors are being used.

Various monoclonal IL-6 receptor inhibitors like tocilizumab and siltuximab, anti-interferon gamma antibodies – Emapalumab, azithromycin, corticosteroids, and IL-1 receptor antagonists - Anakinra were being examined to ensure the efficiency and have proven their efficacy already [79]– [82]. Along with systematic inflammation, other reasons are responsible for heart failure thus, HF increases the risk of sudden death [55]. To mitigate the chance of sudden cardiac death, both chloroquine and hydroxychloroquine can help block potassium channels that can prolong QTc along with azithromycin and lopinavir/ritonavir. Researchers are also working with cell-based therapies to see the effect. CDCs have shown a positive impact to treat heart failure with reduced and preserved ejection fraction [83].

Anti-inflammatory drugs are being used for myocarditis as it is related to systemic inflammation.

According to Minga et al. for reducing mortality rates, β-Blockers can work as one of the foundations of cardiovascular therapy [84] along with anti-inflammatory drugs. Moreover, a study was done with 7 individuals which shows the positive impact of Mesenchymal Stem Cells (MSCs), somatic progenitor cells with immunomodulatory properties [85]. After MSC introduction, patients have improvements in pulmonary function and peripheral lymphocyte count [37]

To treat arrhythmias, the antiarrhythmic drug amiodarone has been used as it is capable of inhibiting the spreading of SARS coronavirus in vitro and now it is being examined for use in patients [86], [92].

A scientific position statement by the Heart Disease Management Program, National Health Mission, Government of Tamil Nadu suggested the use of antithrombotic therapy to minimize various embolism problems [88]. For treating VTE intermediate dose of anticoagulation has been proposed [89] which has been already proved to be helpful in VTE prevention and for managing it during COVID [90].

Oxygen imbalance is one of the most common symptoms in COVID-19. For this kind of hypoxic ICU patient, oxygen therapy is the best possible life-saving therapy yet overabundance of oxygen can cause considerable harm [91].

Azithromycin: It is found to be associated with an increased risk of ventricular tachycardia, cardiac death, ventricular arrhythmias, and sudden death [92]

Chloroquine and hydroxychloroquine: Though this medicine inhibits viral entry it causes QTc prolongation, bundle branch block, AV block, ventricular arrhythmias [40], torsades de pointes [40], [93], cardiomyopathy [94], conduction disorder [95], and direct myocardial toxicity [26].

Lopinavir/ritonavir: Conduction abnormalities can happen because of these medicines [40], the prognosis of atherosclerosis, low-density lipoprotein and total cholesterol to high-density lipoprotein (HDL) ratio, decreased HDL level [96]. Additionally, sinus arrest, the atrioventricular block of first and second degree have been reported [97].

Ribavirin: It causes hemolytic anemia [40], hypotension/hypertension, acute coronary syndrome along with arrhythmias [26].

Corticosteroids: This anti-inflammatory drug can be the reason behind electrolytic imbalance, hypertension [40], and fluid retention [98].

Tocilizumab: Though it inhibits IL-6 and cytokine storm, causes a type of rare hypertension [40], hypercholesterolemia [26]. Moreover, tocilizumab is responsible for decreasing hepatic LDL receptor expression and increasing serum LDL cholesterol [99] which in turn can cause atherosclerotic cardiovascular disease.

Immunoglobulin Therapy: This may be responsible for cardiopulmonary failure [40].

β-Blockers: These blockers harm the cardiovascular system and can cause arrhythmias [84]. With the interaction of lopinavir/ritonavir, β-Blockers can produce PR prolongation and can cause AV block of both second and third-degree [100].

Amiodarone: Can be harmful if it is being used along with lopinavir/ritonavir [93].

Though research manifests various harmful effects on the cardiovascular system by the medicines used to treat COVID-19, detailed studies are compulsory to see the result and to find out other injurious effects of all the medicines.

Chapter 3

3.1 Cardiac Cell Therapy to Treat Myocardial Damage

Studies found that exogenous cell transplantation into damaged myocardium can improve myocardial function and vascular supply [101]–[103]. Transplanting cells to the damaged region for cardiac repair is known as cardiac cell therapy [104]. In situ cellular cardiomyoplasty, cells are being injected directly or intravenously into the infarct [105]. 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. Firstly, fetal cardiomyocytes, and neonatal cardiomyocytes which have been applied in animal studies and have shown improved result. Autologous skeletal myoblasts have also been applied for transplantation as it is highly resistant to ischemia and can differentiate into adult skeletal muscle. Bone marrow stem cells are capable of forming endothelium, skeletal, and cardiac muscle thus used in in-situ cellular cardiomyoplasty. Additionally, embryonic stem cells (ESCs) are also being used as they have the potential of being highly proliferative and totipotent. The transplanted ESC-derived cardiomyocytes are competent in improving myocardial function. Adipose-derived mesenchymal stem cells (ADMSCs) are competent of multipotency like mesenchymal stem cells (MSCs) and they can help in repairing damaged cardiac muscle by angiogenesis and forming cardiomyocyte-like structure. Reports claim that the mammalian heart has the intrinsic regenerative potential and cardiac stem cells (CSCs) are capable of differentiating 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 adult differentiated cells with 4 genes s (Oct3/4, Sox2, c-Myc, and Kfl4) [106] or (OCT4, SOX2, NANOG, and LIN28). A study found that iPSCs can differentiate into smooth muscle, cardiac, and endothelial cells and help to restore contractile performance and ventricular wall thickness in infarcted murine hearts. Cellular cardiomyoplasty uses myogenesis, angiogenesis, and paracrine effect mechanism to do the required task [104].

3.2 Tissue Engineering of Myocardial Tissue to treat Myocardial Damage

Tissue engineering (TE) approaches for cardiac damages have been studied recently. As myocardial tissue lacks regenerative capability so, TE can be a potential alternative for restoring the functionality of damaged myocardial tissue [107], [108]. There are three fundamental elements for a successful TE; cells, extracellular matrix, and biomimetic signal [109]. Cardiac tissue engineering (CTE) contains the synthesis of a scaffold from biomaterial combined with cells that have been grown with additional growth factors [105]. Biomaterial scaffold works as mechanical and biological support for cell attachment, growth, and differentiation. Besides, cellular activities are influenced by growth factors [110]. Additionally, Bioreactors are another important part of making tissue engineering successful. The basic approach is to seed cells that are capable of forming cardiomyocytes onto a scaffold in vitro and then introduce the construct in the infarcted region [105].

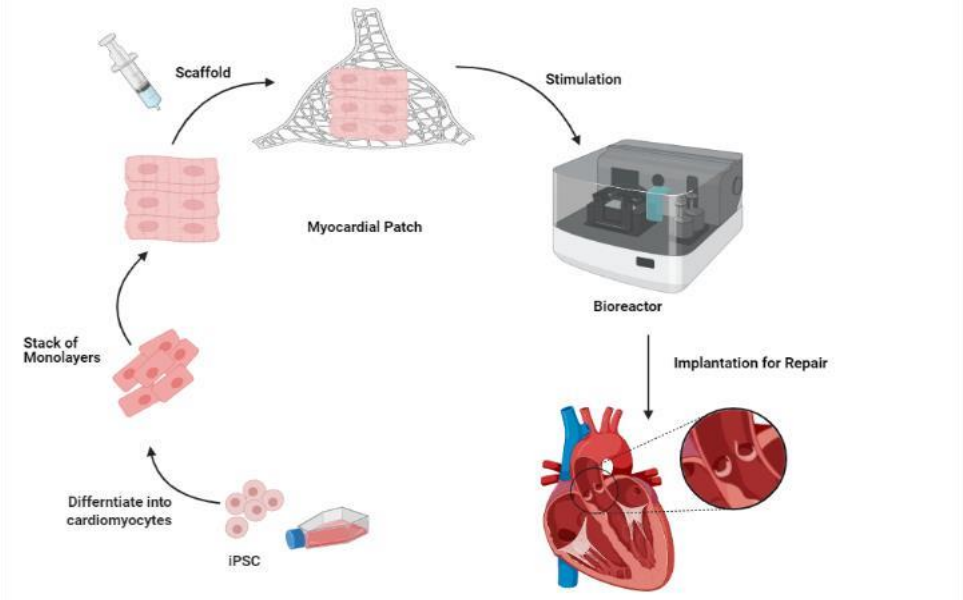

Figure 3: Tissue engineering of myocardium

3.2.1 Cell sources for myocardial tissue engineering:

Selecting the perfect cell for tissue engineering is one of the most important tasks. Only the optimal cell has the capacity of differentiating into mature, and functional cardiomyocytes and will be easy to harvest, nonimmunogenic, and proliferative. The ideal donor cell should contain properties like electro-physical, structural, and contractile which mimic the natural cardiomyocytes. Potential cell sources for myocardial tissue engineering are fetal cardiomyocytes, skeletal myoblasts, mesenchymal stem cells, smooth muscle cells, endothelial progenitor cells, crude bone marrow, umbilical cord cells, fibroblasts, human embryonic stem cells, and cloned cells [110]. All these cells have their capacity to be a potential cell source for tissue engineering. Growing an appropriate number of cells is important to maintain all the specific biological functions, to allow the cells to differentiate into the appropriate phenotype, to interact with other cells/tissues which require extracellular matrix and signaling molecules. Biomaterials help in this regard.

3.2.2 Scaffolds and biomaterials:

Biomaterial scaffold supports the cells not only by providing physical support but also by giving chemical and biological signals needed for the formation of functional tissues [111]. There are certain requirements for all kinds of biomaterials. Biomaterials must be biocompatible, biodegradable, biomimetic, and cell friendly which refers that, biomaterials must increase the adhesion and survival capability of cells both in vivo and in vitro. Besides, biomaterials must be easily available and cost-effective. Mechanical integrity is another important requirement for all biomaterials so that their mechanical properties match with the host tissue and can provide mechanical support. Moreover, biomaterials must resist or help the continuous stretching/relaxing motion of the myocardium [105].

Biomaterials can be achieved from new materials or by chemical modifications of existing materials with bioactive molecules which is more advantageous. Bioactive molecules can be either whole extracellular matrix (ECM) molecules or the ‘cell binding’ doing of those proteins. The selection of peptides depends on the cell type, implantation site, and specific requirements [110].

Both synthetic and natural polymers have been used for scaffold fabrication [112]. Some of the natural biomaterials are Gelatin scaffolds, porous alginate scaffolds, collagen scaffolds, and fibrin glue. Some of the synthetic biomaterials are Polylactic acid-Polyglycolic acid (PLA-PGA), PolyL-lactide-gelatin-PGA, Electrically conducting membrane layers composed of PGA, gelatin, alginate and/or collagen, polyvinyl alcohol, polyurethanes [113], poly(glycerol sebacate), poly(ethylenetephatalate)/dimer fatty acid block copolymer. Both of these polymers have their functionality which can create a better scaffold for tissue engineering. Thus, studies are going on to combine the biological properties of natural polymers with the mechanical performance of synthetic polymers to build a scaffold with better performance [105]

3.2.3 Bioreactor:

Bioreactors stimulate the growth and development of cells or tissues on biomaterials. Bioreactors are capable of producing 3D myocardial tissues consist of more than a few layers of muscle and that makes it more advantageous. Various kinds of bioreactors have been proposed for cardiac constructs. Firstly, static or mixed flask bioreactors; suspends the constructs into a cultivation medium. Secondly, rotating vessel bioreactors; suspends the constructs in a medium that is constantly rotating. Finally, perfusion cartridge bioreactors; here the constructs are continuously being perfused at interstitial velocities [114].

Depending on the scaffold material and cell type that is being used to make the ideal tissue patch for cardiac repair techniques are of various types. We will be discussing a few here:

3.2.4 The Hydrogel Technique

This technique employs hydrogels including fibrin, collagen, alginate, Matrigel^{TM} [115]–[117]. Both the hydrogel (mixture) of interest and the cells are the components of the tissue reconstruction mixture and it is being cast into a mold that can be modified according to the need [136].

3.2.5 Alternative Scaffold-Based Techniques

Synthetic materials like PGA, polyglycerol-sebacate, and polydimethylsiloxane have been used in this technique along with biological hydrogels [120], [121]. Synthetic scaffolds have the capability of creating a defined form and they can initiate functions by cellularization thus it has been used as a cell seeding substrate [122]. Researchers have already tried the recellularization of a decellularized organ like the heart [123] by using the same approach.

3.2.6 The Scaffold-Free Approach

In this approach, exogenous scaffolds are not being used. Instead, cells are assembled in monolayers [122]. These monolayers can be transformed into intact cell sheets by using thermoresponsive surfaces and thus capable of forming a three-dimensional construct [137]. This technology has already been used to deliver skeletal myoblasts to treat heart failure [126] and to deliver allogenic iPSC-derived cardiomyocytes into the human heart [127].

3.3 Limitations/challenges of cardiac cell therapy and conventional tissue engineering

The adult heart cannot regenerate cardiomyocytes after being injured or infracted which leads to regional contractile dysfunction. If the injured area is large, then it can degenerate the remaining myocardium and can lead to congestive heart failure. There are various reports which show the long-lasting cardiovascular effect of COVID-19 on the heart which indicates that the injured area is very large and according to the report, this injury can cause sudden death. Researchers have implemented cardiac cell therapy and conventional tissue engineering technology to solve the problem. However, they had encountered various challenges.

A major disadvantage of tissue-engineered products is the destructive host response. After the introduction of 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 initiate after implantation of the engineered construct. With the combination of all these features, a high chance of graft rejection initiates after the introduction [128].

After doing feasible and safe myoblast transplantation, serious concerns appeared regarding the increased risk of arrhythmias which need to be investigated before future transplantation.

Transplanted cells must transdifferentiate into cardiomyocytes and must survive for a long time to be able to serve the need. However, the infarcted region and the surrounding do not help the cells to survive in the optimal environment which makes the process inefficient.

Collecting the right number of cells for transplantation is also a challenge. Fetal cardiomyocytes were capable of limiting scar expansion and heart failure in rat models. Thus, researchers started working with it and found out the benefits. However, using fetal cardiomyocytes in humans is not a feasible option as acquiring enough human fetal tissue itself is a challenge [129]. Rodrigues et al. have mentioned the advantages and disadvantages of all the cells that can be used in cardiac cell therapy. Different cells can have different disadvantages. 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, risk of myocardial complications is associated with most of the cells that are being used in the process [130].

Myocardial tissue-engineered constructs use multiple defined and undefined cells which support sin 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 [122].

Mantakaki et al. present the advantages and disadvantages of the materials and biomaterials that are being 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 life long 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 [131].

According to Wang et al., other limitations of tissue engineering are the inconsistence 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 [104].

Engineering large size cardiac constructs through tissue engineering is a challenge as cardiac tissues are composed of ECM proteins and different cells with different alignment to make spontaneous contraction possible. All the properties of the native myocardium cannot be maintained throughout. As a result, the construct is unable to contract like the native one and the cells died after a few times [132].

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 very challenging to fabricate in a way that it will be functional for a longer period because of the huge number of branched blood arteries and capillaries [132]. Moreover, seeding cells on these scaffolds lead to cell death and can cause poor cellular performance [133]

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 [132].

Chapter 4

4.1 An alternative to treat myocardial injury “3D Bioprinting”

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 is capable of holding control over the structure of the cell-biomaterial architecture, provides the required physiochemical 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. To make a 3D structure that mimics the actual organ/tissue, a perfect bio-ink should be selected for the fabrication. To achieve the perfect bioink, the mixture of different biomaterials based on the mechanical properties, acquire attention. [133]. 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.

4.1.1 Bioprinting Process

The whole Bioprinting procedure can be divided into three distinct stages. The first one is the preprocessing stage where all the planning for successful bioprinting is present. Imaging is a must to analyze the overall structure which will help in making the blueprint for bioprinting. The second phase is Bioprinting. All the complexity is present in this stage. Choosing the right bioprinting technology along with the appropriate bioink and cell is a tricky job as all these things can manipulate the effectiveness of the printed 3D construct. Moreover, the bioink 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. 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 [134].

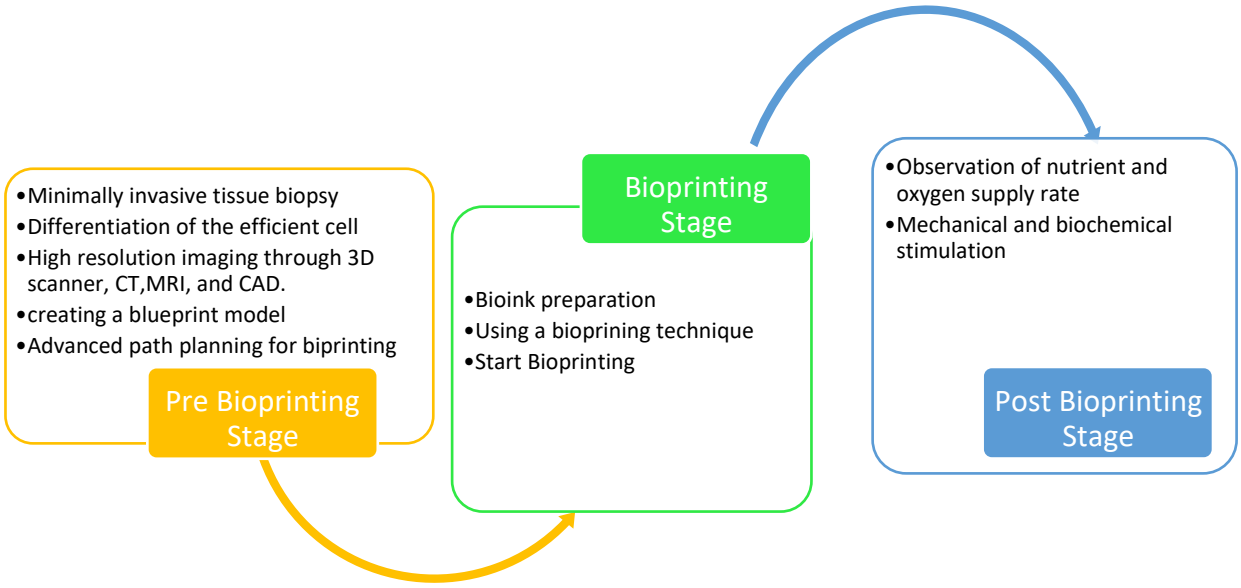

Figure 4: Schematic diagram of 3D bioprinting

4.1.2 Bioinks

Bioinks is the first and foremost component to make the 3D construct perfectly. They need to maintain some important characteristics like biocompatibility, printability, mechanical and structural integrity, biomimicry, and biodegradability. Bioinks can be divided into two broad categories. Those are natural polymers and synthetic polymers.

The natural bioinks are isolated from natural sources. They have the potential to improve the biological features of the printed construct. Cellular behavior like migration, proliferation, differentiation, and maturation can be achieved by natural polymers as they provide tissue-specific biochemical and physical stimuli. However, the natural polymers lack mechanical stability,

structural mimicry from batch to batch. Based on the protein composition, natural polymers can be of various types. Such as collagen, gelatin, fibrin, silk, polysaccharides, etc.

On the contrary, synthetic bioinks can stimulate the mechanical integrity of the 3D construct. Some synthetic polymers can hinder the adhesion of cells and can be responsible for cell death. Thus, choosing the right synthetic polymer is a must. The best option can be polyethylene glycoldiacrylate and polyethylene glycol.

Moreover, chemical conjugation of synthetic and natural polymers can be a better option to work with. For example: gelatin-methacrylate (GelMA) or PEGylated gelatin methacrylate (PEGgelMA) [135].

4.1.3 Types of cells for 3D bioprinting of Cardiovascular tissue

Cells are capable of forming tissue if the exogenous factor, like neighboring cells, biomaterials, and biofactors is suitable for the cluster formation. Therefore, the supply of proper cells is the most important task. In this case, cells are obtained from autologous tissue and autologous or allogeneic stem/progenitor cells. Stem cells have various advantageous characteristics which help them to differentiate in multiple cardiovascular lineages. Human common myeloid progenitor stem cells (hCMPCs) and human induced pluripotent stem cell-cardiomyocytes (hiPSC-CMs) are popular choices. Both of the cells need to be obtained from patients so that, the cells can present the genetic, environmental, and physical differences of individuals. Studies have already demonstrated the benefits of using these cells for cardiac treatment [132], [135].

4.1.4 3D bioprinters for Tissue Engineering

3D bioprinters are commonly classified into four common categories. However, seven types of bioprinters are there. Based on the structural properties of the selected tissue/organ, we need to choose the bioprinter. The seven types of bioprinters are (1) inkjet-based, (2) extrusion-based, (3) laser-assisted, (4) stereolithography, (5) acoustic, (6) microvalve, and (7) needle array bioprinters.

Here, the working principle of each printing module along with the fundamental characteristics are given.

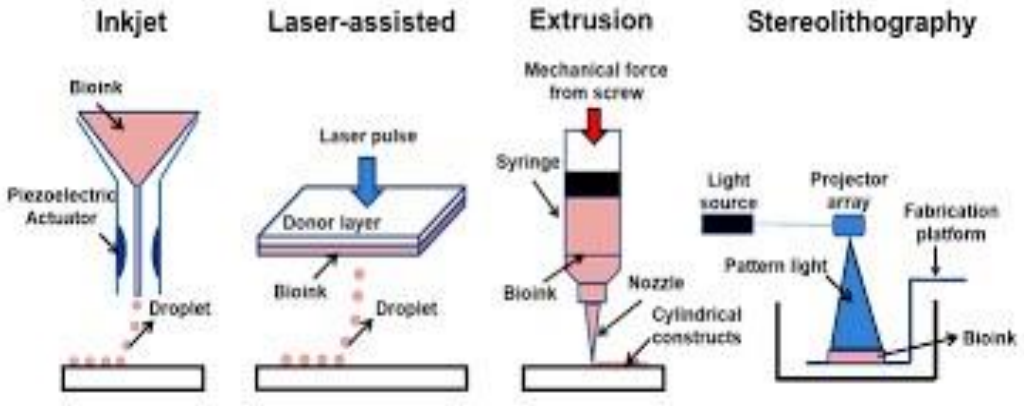

Figure 5: Four main categories of 3D Bioprinting technology

4.1.4.1 Inkjet-Based Bioprinters

Inkjet printing is also known as drop-on-demand printing [137]. It is a high-resolution printing procedure as it can create very small droplets with the help of mechanical vibration of the printing head. The bioink that is being used in this system must be of lower viscosity. Two methods are used to bring down the bioink through the head. Those are the thermal method and the piezoelectric method. The generation of specific structures is facilitated by the motion stage. A microheater adjacent to the printing nozzle elevates the temperature which helps to create drops of bubbles and these bubbles are printed as droplets in the thermal inkjet printer. On the other hand, a piezoelectric actuator of a piezoelectric inkjet printer generates a direct mechanical pressure to expel bioink droplets from the printing head. Here, the diameter of each droplet depends on the nozzle size [135] [133]. Inkjet-based printers have a high printing speed, relatively low cost and it is a high throughput technique with a high precision rate. Thus, they are highly being used to print skin, cartilage, bone, and blood vessels [137].

4.1.4.2 Extrusion-Based Bioprinter

Extrusion-based bioprinters are capable of handling high cell density, viscosities, and crosslinking mechanisms. As they are capable of using biomaterials of different viscosities, so biomaterial including synthetic polymers, cell-laden hydrogels, cell aggregates, and microcarriers can be used [133]. These printers utilize various forces like pneumatic, mechanical, or solenoid-induced forces to expel the bioinks to fabricate a 3D architecture. To improve the printing process various advancements have been done. For example, the production of temperature-controlled nozzle or stage system, multiple direction-controlled nozzles, and independently controlled nozzles. Extrusion-based bioprinters are mostly being used to fabricate cardiac tissue constructs as these printers can deposit relevant cardiomyocytes in the engineered construct, can utilize various types of bioinks, high-speed bioprinting capability, ease of operation, and affordable enough compared to other bioprinters that are available [138].

4.1.4.3 Laser-Assisted Bioprinters

Laser-Assisted bioprinters contain an energy-absorbing layer, a donor ribbon, and a bioink layer. Firstly, a laser is illuminated on the donor ribbon layer which leads to the creation of a highpressure bubble. Then this bubble presses the bioink layer to generate droplets and can be deposited on the substrate. These printers have various advances including, low contamination risk, highviscosity bioink deposition, nozzle free printing procedure. However, the chance of cell damage due to laser intensity, costly printing modules, harder operating system are the disadvantages of these printers [133].

4.1.4.4 Stereolithography Bioprinters

Compared to other bioprinting methods, this system utilizes light to crosslink the bioinks in the reservoir using a layer-by-layer procedure. Thus, this system can only work with light-responsive

bioinks, like gelatin methacrylamide (GelMa) and polyethylene glycol diacrylate (PEGDA). Moreover, there is a high possibility of having infused reservoirs with the photopolymers which cause wastage of materials and can make the experimentation costly [133].

4.1.4.5 Additional Bioprinters

Acoustic and microvalve bioprinters are considered droplet-based bioprinters. Acoustic waves have been used to eject droplets from acoustic bioprinter, and microvalve bioprinters utilize electromechanical microvalve where the magnetic field is generated by the valve coil and it leads the plunger upwards, and finally, the droplets are being ejected from the bioprinter. Needle array printers use the scaffold-free method, which makes a string-like structure with the preformed cell aggregates onto a needle array platform [133]

4.1.5 Hybrid Printing Strategies

Each of the 3D bioprinting modules has some unique characteristics and these can be combined depending on the required characteristics to generate a complex tissue construct. Investigations have been done to develop bioprinters with multiple printing modules to generate a complete structure. Such as integration of an inkjet-based bioprinter with electrospinning, a laser-assisted bioprinter combined with electrospinning, the combination of inkjet-based bioprinter and extrusion-based bioprinter, the combination of extrusion-based bioprinter with electrospinning. A study claimed that a combination of inkjet-based bioprinters and electrospinning helps to elevate the mechanical strength of the cellular construct while doing cartilage tissue engineering. Moreover, a hybrid bioprinting system that can utilize inkjet-based bioprinting modules and extrusion-based bioprinting modules simultaneously is suggested to fabricate 3D in-vitro kin models. Here, the structures are capable of doing better mechanical and biological activities compared to conventional 3D bioprinting [133].

4.2 Applications of 3D Bioprinting

4.2.1 Organ/Tissue regeneration:

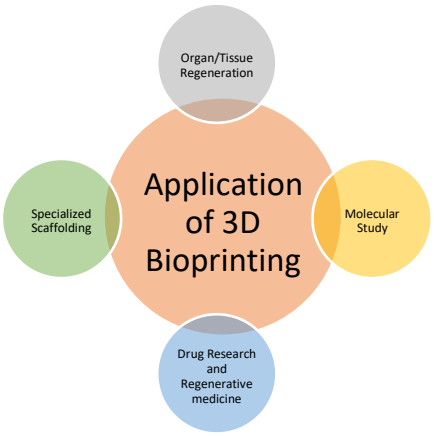

Figure 6: Application of 3D bioprinting

The number of organs needs for transplantation is huge compared to the number of donors available. Thus, developing functional, fully-sized organs are needed. 3D bioprinting has great potential in this field to create a biomimetic fabrication of vascularized tissue. Organs like the brain, heart, lung, and kidney can be created through this process. However, research is still going on to make this process more precise [139]. Successful tissue fabrication of bone, cartilage, blood vessels, liver, osteochondral tissue, neuron tissue, cartilage, trachea, and so on [133].

4.2.2 Molecular study

To know about a certain disease or complication, the overall molecular study is very important. 3D bioprinting is a solution to do so. Patient-specific 3D constructs are helpful to analyze all the molecular basics. This is possible for the most complex construct heart as well. Molecular study of cardiac functions, exploring signaling pathways will be helpful to predict the therapeutic/toxic responses accurately [138].

4.2.3 Drug research and Regenerative medicine

After detecting a new disease, a race starts in the pharmaceutical industries to screen the effective drug and deliver it. In different clinical phases, the drugs are being studied to understand the effectiveness of the drug. Traditional 2D or 3D cell culture systems or animal models cannot provide accurate in-vivo results in humans as these systems cannot recapitulate the exact physiological conditions of the complex organ systems. 3D bioprinted organs, (organ-on-a-chip) are capable of mimicking the actual organ environment thus help to analyze drug toxicity/therapeutic responses. For efficient drug molecule analysis, a biosensor which is a muscle powered, biological, microelectromechanical system (Bio-MEMS) is being bioprinted recently and it is showing positive results. An interesting example is the screening of the effectiveness of the anticancer drug doxorubicin. The screening was done on endothelialized-myocardium-on-a chip which shows important results and thus this system can be beneficial to determine drug induced cardiovascular toxicity. Moreover, it can open the way towards personalized drug screening in the future [138].

4.2.4 Specialized scaffolding

Researchers have concentrated on the use of 3D bioprinting for skin treatment and regeneration. Skin tissue has been printed already through laser-based direct-write cell printing technology. To analyze the result, they have developed a bilayer skin containing 20 layers of fibroblast-containing collagen and 20 layers of keratinocyte-containing collagen and placed on the wounds of nude mice in-vivo. The functional skin was fully connected with mice skin after 11 days and it gives a positive hint to work in developing bilayer functional skin which mimics human skin [140].

4.3 Why 3D bioprinting is a better alternative for treating myocardial injury associated with COVID-19

In comparison to conventional tissue engineering techniques, 3D bioprinting is capable of influencing 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 the ability of 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 very important for the overall potential of the 3D bioprinting process which makes the technique better than the rest [134].

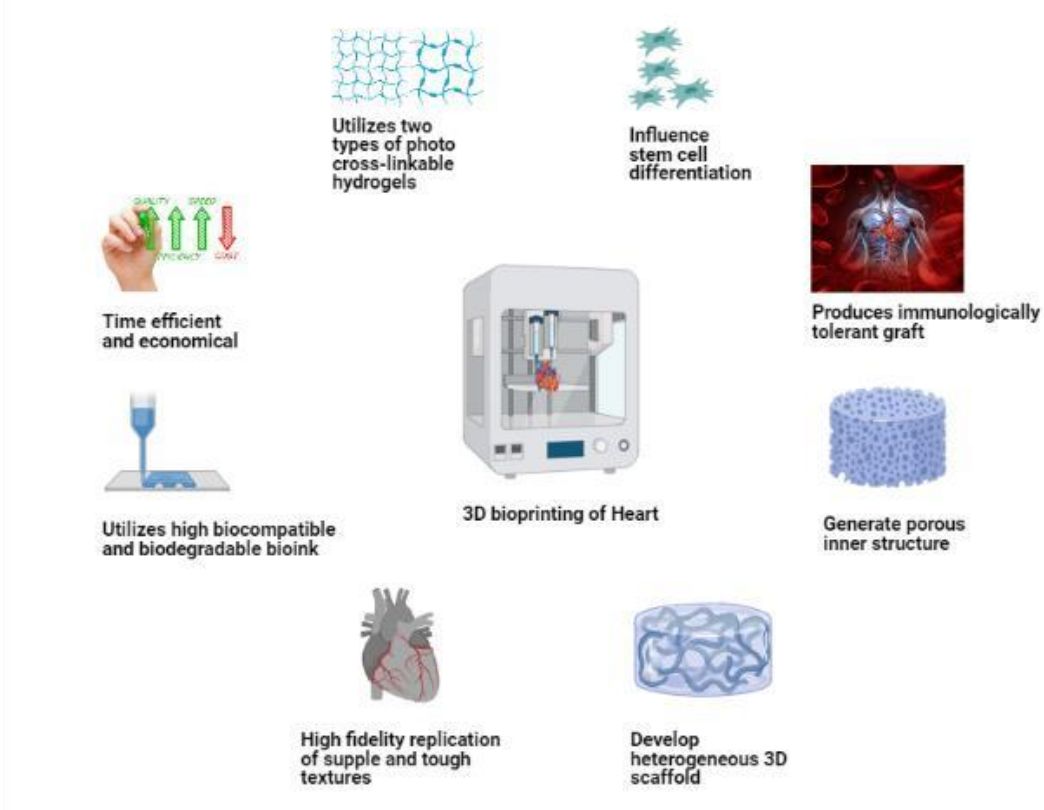

Figure 7: Advantages of using 3D bioprinting

Generally, the other regeneration techniques emphasize more on the shaping capability of the ink. On the other hand, the 3D bioprinting technique focuses on the production of high biocompatibility and /or biodegradability of the bioink to help the merging of the living cells or bioactive molecules. The use of hydrogel dECM has great potential in 3D CTE bioink preparation. Cardiac dECM is capable of reproducing the exact 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.

Another advancement of 3D bioprinting which makes this technique a better alternative is the high fidelity replication 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 [142].

The 3D bioprinting process utilizes two types of photo cross-linkable hydrogels, one of which is rigid, works for the root and another is soft, which works for the leaflets. The printing procedure was extrusion-based and within 45 minutes the heart was printed which indicates that 3D bioprinting is a speedy technique compared to any other technique out there for myocardial regeneration [141].

3D bioprinting is capable of developing heterogeneous 3D scaffolds of strong mechanical strength that poses 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 [132]. Moreover, this process has precise control over, various compositions, structural complexity, distribution, effective printing of all the tissues with accurate features [138].

3D bioprinting is a promising approach to generate porous inner structure which helps to provide the required nutrients and oxygen to other tissues [135]. As the technique is automated, mass production of cells is easier with it and the construct will be of high resolution [133]. Additionally, the 3D products contain precise architecture which is reproducible and repeatable [139].

This technique uses tissue-specific models for bioprinting the organ or certain 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 information of genome, proteins, and medical/family history which will help reduce life-threatening effects [135].

As the 3D bioprinting technique is capable of regenerating customized and complex 3D models of human tissues and organs, it is beneficial to reduce the number of animal testing and can overcome all the ethical concerns related to animal testing [143].

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 [132].

Chapter 5

5.1 Discussion

The chance of having myocardial complications in COVID-19 patients is huge. Thus, all the recovered patients need to be under observation for a certain period to analyze the effect. Different patients will have different types of problems. The chance of sudden cardiac death arises when the patients have pre-existing heart complications. Moreover, patients with diabetes, high blood pressure, history of heart attack have a higher chance of death. There is a high chance of having another pandemic situation where all the recovered COVID-19 patients will be having heart complications at the same time. Reports show that many recovered patients are having heart complications after a few days of recovery. This is a clear indication of the long-term effect of the disease. It is necessary to make the recovered patients aware of the upcoming problems so that they can be careful and take medical consultation immediately.

The drugs that are being used for treatment, are capable of damaging the heart cells even more. Some medicines can cause great damage while being used together. It is necessary to understand the physical condition of the patients before using the medicines. As this disease is completely new, so research must go on to understand every aspect of it. Different reports have already been published which talk about different kinds of responses to the same medicine on different patients. These reports can be beneficial to understand the effect before using them.

Damaged tissues of the heart must be replaced to help it work properly. Currently, there are techniques like cardiac cell therapy and cardiovascular tissue engineering is present to solve the problem of tissue damage. These techniques can generate new tissue to be replaced with the injured one. However, these techniques have so many limitations which make them incapable. There is a huge chance of rejection in these techniques because of the cell source being used here. If the hist body rejects the graft, a new engineered tissue needs to be placed in the injured region. As a result, these techniques become costly.

In the case of 3D bioprinting, various bioprinters can be utilized. Depending on the cell type and some other factors, the bioprinting technique is selected. Among all the techniques, the inkjet

technique and extrusion-based bioprinting techniques are better for myocardial tissue regeneration. Here, the printed portion becomes so precise and mimics the native myocardium. The functionality of the printed tissue/organ remains similar to the actual tissue/organ. Moreover, the printing speed is higher than the other that makes the technique more attractive. Thinking about the cost, there is less chance of rejection by the host immune response as the host cell is being used to produce bioink. Thus, the cost of the whole procedure remains less.

Despite having so many reasons for using the technique, there are some limitations and challenges while working with this technique. Those are given below:

The functionality of a construct mostly depends on the control over the organization of the vascular system. As cardiovascular diseases are related to the perfusion of oxygen and nutrient through the vascular network, advanced vascularization techniques are required to construct physiologically functional tissues. Though the 3D bioprinting technique is capable of mimicking the natural construct, studies are going on to achieve the whole vascularization control. Researchers have suggested the incorporation of cells or biofactors in the engineered construct to enhance the vascularization of the newly made construct and it will help in long-term tissue survival [135].

To make 3D bioprinting successful, another challenge is the selection and preparation of the perfect bioink. The bioink must pose all the required characteristics and it must be maintained invivo for a longer period. Though there are various bioinks present to use in 3D bioprinting, researchers are working continuously to overcome the current limitations and trying to develop a perfect bioink that contains a balanced bio-printability and bio-functionality [133].

As the bioprinting technique needs bioactive polymers for the procedure, sometimes the selected molecules are biologically so active that they can cause unwanted cellular interactions and unfavorable stem cell differentiation. Thus, choosing the appropriate biomolecule can be a challenge [134].

For effective industrial translation and commercialization of 3D bioprinted organs, quality assurance and regulation of bioinks, bioprinters, and bioprinted products are a must. This can be a challenge. Maintaining the quality in all the steps of 3D construct regeneration can be a little harder. The guidance issued by FDA must be maintained. Ethical concerns can also cause problems while implanting the 3D bioprinted construct in humans [139].

5.2 Conclusion

In conclusion, this review commences with the complications of heart associated with COVID-19 which is caused by SARS-CoV-2. The heart ACE2 helps the virus to enter into the heart of an individual and cause problems along with lung. The damage that is caused by the virus is irreversible. Moreover, the medicines that are being used can cause great damage to the cardiovascular system. To replace the damaged heart with a new one, techniques like cardiac cell therapy and cardiac tissue engineering can be utilized. However, various limitations make these techniques impotent. The cases of COVID-19 infected individuals and death from this disease are increasing at an alarming rate. Moreover, the symptoms and complications are getting different from the very first case day by day because of the mutations that are happening in the viral structure. Additionally, the type of cardiovascular damage is changing. These complications are capable of creating another pandemic. To solve the upcoming problem, 3D bioprinting shows huge potential as it will be helpful to generate a functional, biocompatible tissue, and if the damage rate is higher than it can print the whole organ and replace it with the damaged one. Studies are still going on to make this technology more appropriate so that it can generate a heart that mimics the native heart and all its features and solve the upcoming problem with accuracy.

Chapter 6

6. Reference

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