# Utilizing Regenerative Medicine and Tissue Engineering to Improve Treatment of Advanced Heart Failure

Image Source: thecardiologyadvisor.com


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# Abstract

Heart failure is a debilitating cardiovascular disease that results from ischemic events such as myocardial infarctions (heart attacks), plaguing people for years with no known cure. The most viable treatments for HF are limited to LVAD implantations and heart transplants; however, such treatments are incredibly costly, and they still have implications after being implemented. To treat HF before such expensive and invasive measures are used is the objective of this document. Recent advances in stem cell research and 3D bioprinting provides promising avenues to address the challenges physicians face when treating HF. The document analyzes the fundamental problem associated with HF, which is regenerating cardiomyocytes lost after MI and subsequently rehabilitating cardiac function. The proposed solutions to address this problem include cellular-based engineering, 3D bioprinting, and scaffold-free tissue engineering. Scaffold-free tissue engineering provides the most promising outcomes in treating HF in terms of its cost, effectiveness, and invasiveness when compared to the other solutions. If implemented, the solution could be made available to a large population of patients who cannot afford current HF treatments while simultaneously providing an effective treatment.



**Keywords**: Heart failure, HF, cardiomyocytes, CM, induced pluripotent stem cells, iPSC, human embryonic stem cells, hESC, 3D bioprinting, scaffold-free tissue engineering


**Document scenario:** This document proposes an engineering project to remedy heart failure in patients before invasive treatments are utilized. The document is envisioned as a proposal submitted to government organizations such as the National Institutes of Health (NIH) and the American Heart Association (AHA). In addition, this proposal could be submitted to private hospitals, various research institutes, or biotechnology companies. Researchers, cardiologists, and biomedical engineers would primarily review the technical proposal. The Executive Summary would be read by officials from the NIH and/or business managers who would fund the project.

## Executive Summary

Heart failure (HF) is a chronic cardiovascular disease that affects about 6 million of Americans, with related healthcare costs reaching almost $31 billion (CDC.org, n.d.). Thus, heart failure and its resulting medical expenses are a significant burden to HF patients. With no known cure, the current treatments to treat advanced HF resort to invasive measures, such as implanting LVADs and heart transplant surgeries. Heart transplants in particular are not ideal; aside from the costs associated with it, there are not enough donor hearts available for everyone who needs a new heart leaving many to suffer from their condition, and there are often post-transplantation implications that require even more visits to the hospital. The proposed project aims to treat HF before expensive, invasive treatments are used while simultaneously providing patient-specific treatments.

Utilizing cell-based therapies and tissue engineering on animal models with heart injuries have been shown to rehabilitate cardiac function. However, such treatments are still in its early stages of development, and key design requirements and constraints must be considered. The proposed candidate solutions are assessed in terms of effectiveness, invasiveness, delivery method, and materials/resources needed. Ensuring that these design requirements can be fulfilled is imperative for the project's objective to not only regenerate cardiac function, but to also be less expensive than current treatments and more patient-specific. The design of each solution differs in many aspects and how they are developed, but there is slight overlap among them.

Scaffold-based tissue engineering is the most viable solution to provide a personalized approach when treating advanced HF. This solution is essentially a bridge between 3D bioprinting and cellular-based therapies. It will not require the use of external biomaterials and associated outsourced resources; it only needs the patient's cells and an orbital shaker to create a tissue that rehabilitates cardiac function. The tissue itself can be controlled in terms of size, thus enabling physicians to target specific areas of the patient's heart that needs critical attention, rather than wasting resources to create a one-fits-all cardiac tissue that might not be effective for every patient. The success of the tissue after implantation reveals that this solution is efficient and minimizes invasiveness and immune rejection, while also maintaining the rehabilitated cardiac function.

If implemented successfully, this project has wide-reaching impacts on heart failure patients on a global scale. Patients who are reaching end-stage heart failure will no longer need to wait for a donor heart, allowing physicians to treat more patients. Not only does this solution provide a lower cost alternative to current treatments, but it also provides a treatment that directly focuses on each patients' needs and uses their own cells to help their heart heal efficiently; successful implantations of the tissue will require less hospital visits, travel, etc. which saves time and money for patients. HF is an economic burden on the government as well; healthcare programs that provide funding for HF treatments can reallocate funds to other government-funded organizations that focus on improving public health. Furthermore, this solution could potentially be implemented to treat other organ failures.

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# Problem Analysis

This section defines the issue of heart failure and its effect on myocardium. Traditional solutions will also be examined, as well as project constraints.

## Overview of problem and its significance

Heart disease is the leading cause of death worldwide, and it embodies several conditions that affect the heart's structure and function. Cardiomyopathies involve the myocardium, or the heart muscle. Heart attacks occur when there is a lack of oxygen brought to the heart muscle as a result of coronary arteries becoming narrowed from a buildup of fat and other substances, thus reducing the blood flow that carries oxygen to the heart (heart.org, n.d.). When the heart becomes deprived of essential nutrients and oxygen from the blood, the heart is said to be ischemic. If ischemia results in damaged heart tissue, it becomes a heart attack, or myocardial infarction (MI). The severity of MI can range depending on the time between the injury and treatment, and the damaged muscle will begin to heal through the formation of scar tissue. If there is significant damage, the heart will be weakened and have much more difficulty pumping blood.

For people who suffer from end-stage heart failure (HF), they will need to have heart transplant surgeries. While the outcome of the transplantation can result in improved quality of life, the ability to receive a healthy, suitable donor heart is extremely difficult, as patients will have to wait a significant amount of time before receiving one. Even if a suitable heart is found, the transplantation may not be successful because the immune system of the patient may reject the foreign organ, either immediately or over a longer period of time, leading to organ failure or other illnesses. Studies indicate that there is 72.5% 5-year survival rate, and an 84.5% 1-year survival rate (Wilhelm, 2015). While these rates have increased in recent years, the provided statistics, and the impact of heart disease on people worldwide, indicate that HF treatment is an urgent need that must be attended to.

## STEM fundamentals of problem

The heart is comprised of three layers: the endocardium, **myocardium**, and epicardium, and it is one of the first organs to be formed during the embryonic stage of development. The myocardium is the thickest layer of the heart, and it consists of **cardiomyocytes (CMs)**. CMs have the crucial role of coordinating with other CMs to establish the contraction of the heart to provide adequate blood/nutrient supply throughout the body. Infections of the myocardium results in the weakening of the heart muscle, consequently reducing the ability to pump blood (Tran & Lopez, 2020). The cardiomyocyte's limited capacity to regenerate is an obstacle that researchers face when treating cardiovascular diseases. During the embryonic stage of development, **stem cells** are produced. These unspecialized cells have the ability to limitlessly self-regenerate, and can differentiate into various specialized cell types, such as cardiomyocytes, neurons, and blood cells (Hosoda et al., 2011). Figure 1 displays how a pluripotent stem cell can differentiate into several types of CMs. Once the cell is differentiated, it cannot become undifferentiated. Thus, the heart is considered to be a terminally differentiated organ because it is composed of highly specialized cells.

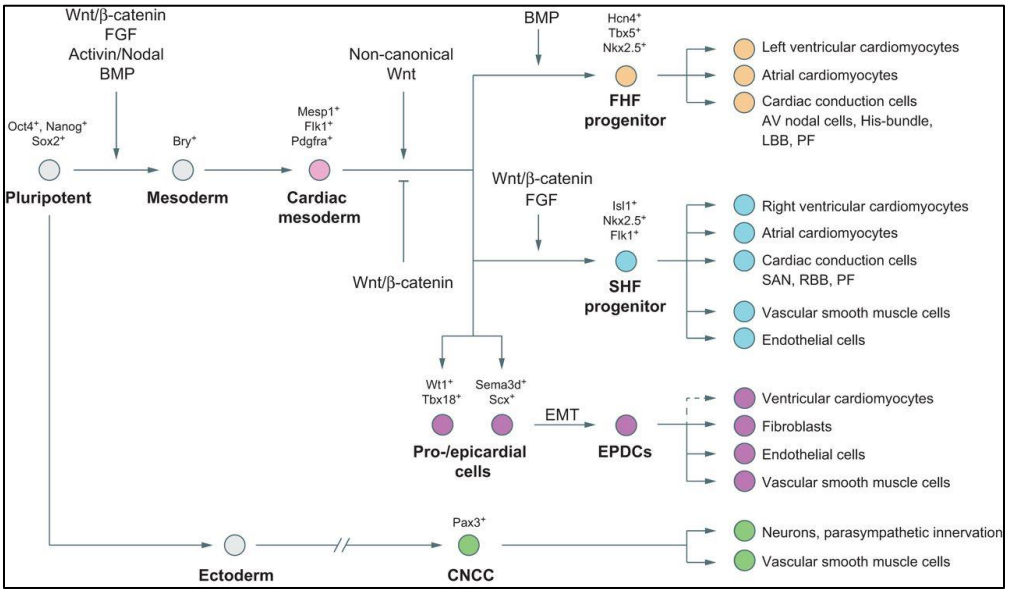


**Figure 1. Differentiation of cardiac cell types during development**

Source: (Später et al., 2014)

It is important to note that regeneration refers to reconstruction of the damaged structure to retain the same cell number and pattern (Hesse et al., 2018). In mammals, the ability to repair/regenerate cardiac tissue depends on the developmental stage, as seen in Figure 2. When observing the embryonic and neonatal stages of development, CMs retain the ability to regenerate successfully to an extent after injury/lesion. However, the adult developmental stage differs because it loses the ability to regenerate CMs completely, and injuries such as MI result in a permanent loss of CMs; cardiac repairs at this stage involve **hypertrophy**, but hypertrophy does not form the original structure. Instead, hypertrophy involves increase in size of the remaining CMs to to make up for the lost CMs during injury. Thus, the heart loses functionality

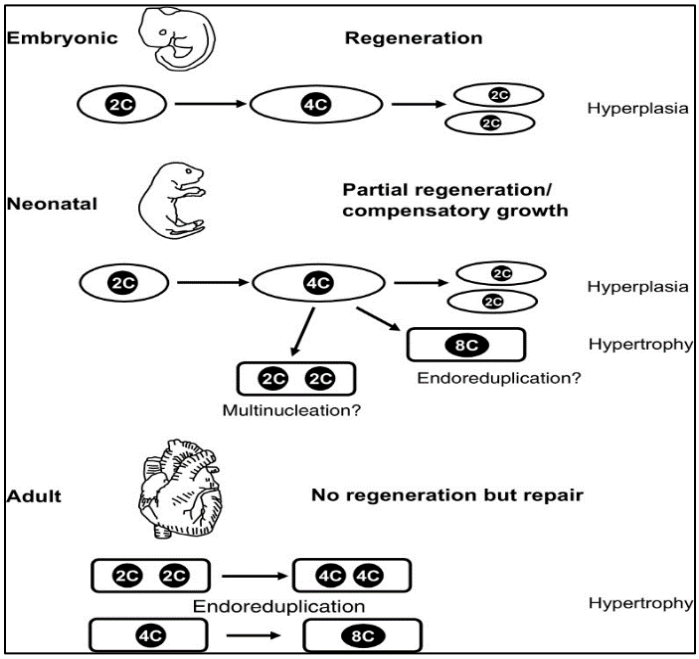


**Figure 2. Cardiac cell regeneration at different developmental stages**

Source: Später et al., 2014

and the myocardium experiences abnormal enlargement that leads to inefficient pumping of blood.

The inability to regenerate CMs at the adult developmental stage is due to the reduced CM cell activity during postnatal heart growth. At this stage, the expression of cyclins and cyclin-dependent kinases, which are responsible for initiating mitotic processes, diminishes. Simultaneously, the expression of cyclin inhibitors increases, which promotes the obstruction of the CM cell cycle. Thus, adult CMs rarely exhibit any cell cycle activity. If any cell cycle activity occurs after lesion, most of the CMs will experience cell cycle variants, such as endoreduplication, and will not divide; such variants lead to hypertrophy (Hesse et al., 2018). Thus, the CM's extremely limited ability to regenerate leads to complications after being damaged.

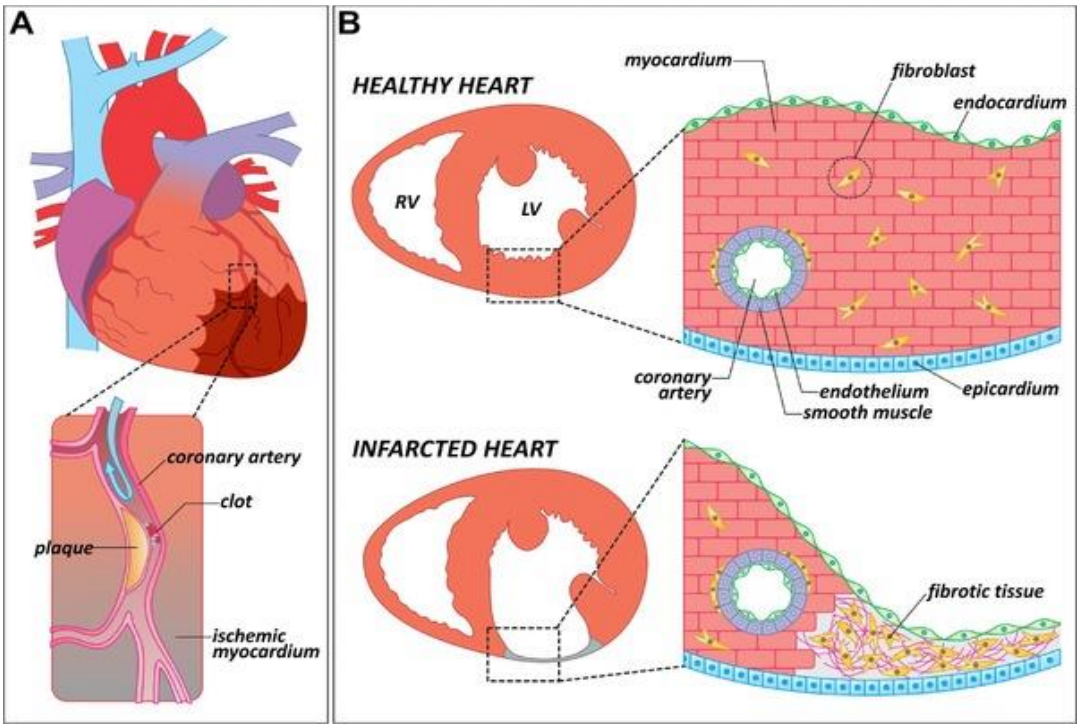


**Figure 3. Schematic representation of a healthy heart and a heart that suffered from MI, resulting in the formation of fibrous tissue**

Source: González-Rosa et al., 2017

The injured/dead CMs are substituted with non-contractile fibrous tissue, which leads to functional weakening and subsequently to the development of heart failure (Kadota et al., 2020). Figure 3 provides an illustration that compares healthy heart muscle to a heart muscle that was affected by MI. The figure displays how fibrous tissue forms when there is a significant loss of CMs. Various engineering techniques must be implemented to address this fundamental problem.

## Lessons from prior responses to the problem

Left ventricular assist devices (LVAD) are used for patients who have reached end-stage HF. These devices are battery-operated mechanical pumps that are surgically implanted into the patient, and they work by assisting the left ventricle in pumping blood to the rest of the body when the heart is ineffective in pumping blood on its own. Today, LVADs are portable and can be used for months while waiting for a heart transplant.

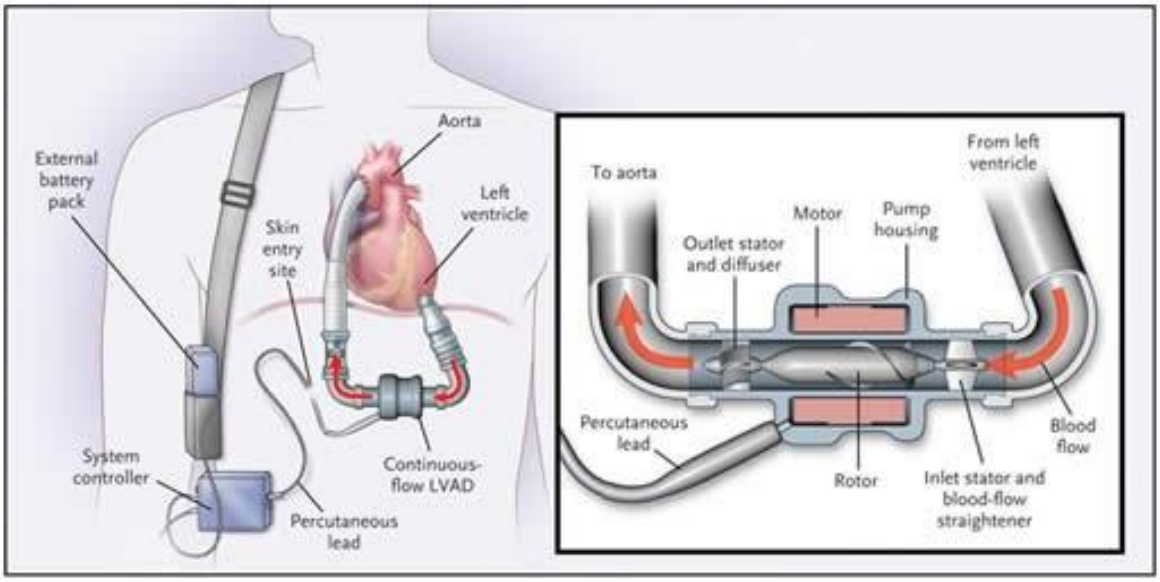


**Figure 4. Schematic representation an LVAD**

Source: stanfordhealthcare.org

An LVAD can be used in two different scenarios: bridge-to-transplant therapy and destination therapy (heart.org, n.d.). Bridge-to transplant therapy is intended for HF patients who are in desperate need for a heart transplant; the patients are implanted with the LVAD until there is a suitable heart for them for transplantation, and in rare cases, the LVAD is able to rehabilitate the injured heart, consequently eliminating the need for heart transplantation. Destination therapy is used for patients who are not eligible for heart transplants, in which case the LVAD can be used to help them achieve a longer life. While the 1-year survival rate for LVAD implantation is about 80%, there are still various difficulties associated during the surgery as well as after (Kadakia et al., 2016). The most common complication is bleeding, which predominantly occurs in the gastrointestinal tract. Aside from internal bleeding, infection, stroke, and thrombotic complications also occur. These complications result in readmissions to hospitals, which increases costs for patients.

Heart transplantation remains the only viable option for end-stage HF. With this treatment, the surgeon replaces the injured heart with one taken from a donor who has been declared brain dead. While 1-year survival rates have improved dramatically since the inception of the treatment, heart transplants still face significant technical problems. First, the process of finding a suitable donor heart often takes months because the donor heart must meet certain standards when compared to the patient's heart; the blood type, body size, and tissue of the donor heart must closely match those of the patient. Furthermore, the amount of heart transplant

candidates substantially exceeds the number of viable donor hearts, inevitably leaving many people on the waitlist to suffer from their condition (Poston & Griffith, 2004). In addition, the patient will need to take immunosuppression medication to prevent their immune system from rejecting the donor heart; however, such medication can lead to adverse side effects such as infection or cancer (heart.org, n.d.). After the surgery, the patient will need to be closely monitored by doctors to ensure that the newly transplanted heart has not been rejected, which can be seen in the cardiac cells. The side effects from the immunosuppression medication must also be watched closely to ensure patient safety.

## Project objectives and constraints

The objective of this project proposal is to provide different treatment options for individuals suffering from HF as a result of diseases and injuries such as MI. These treatments are focused on repairing and regenerating damaged myocardium to restore and retain heart function. Because heart transplants and LVADs are seen as last-resort options for end-stage HF, the proposed solutions will attempt to treat the heart before patients are put at risk of complications during and after LVAD/transplant surgeries. Since current treatments often involve further complications post-transplantation, this project aims to produce the same, if not higher survival-rates after the solution is implemented to indicate that heart function is preserved. Upon reviewing the different proposed solutions, this proposal will review the technical aspects of each solution and find the most appropriate therapy to treat HF.

When considering these potential solutions, one must consider any possible constraints. General constraints when looking at cell-based therapies include maintaining the number of implanted cells and cellular survival, while ensuring that the delivered cells do not stray from the target sites of the organ. Thus, developing these treatments must be carried out in an appropriate lab setting with appropriate biosafety cabinets, equipment, and incubators. Furthermore, producing the cells must be carried out at a large scale while still maintaining clinical integrity. For tissue engineering, researchers must understand that the heart is structured in a complex manner when observing the tissue formation of the heart. Thus, constraints include selecting cell sources and biomaterials that are suitable for engineering new tissue, as well as structural characteristics. In addition, the associated physiological functions must be maintained (Cui et al., 2018).

Along with physical constraints, regulatory constraints must be considered. The FDA oversees the regulation of biotechnology; thus, the proposed solutions must comply with the standards constituted by the agency; stem cell-derived therapies must submit an Investigational New Drug (IND) application before being put to use. In addition, donors of the cells must give written consent after being informed of risks. EPA standards must be met when developing treatments so that biohazardous waste produced during the production of treatments is properly disposed of. In addition, any production of hazardous waste during development must also comply with the Occupational Safety and Health Administration (OHSA) and the Department of Transportation (DOT). As a result, the proposed solutions must be mindful of regulatory constraints and utilize resources efficiently to produce as little hazardous waste as possible.

## Candidate Solutions

This section of the document encompasses the scope, explanation, and comparison of the three proposed solutions for treating HF. These engineering solutions are assessed in terms of design, effectiveness, invasiveness, and cost.

### Scope of solutions considered

While human hearts lack the ability to regenerate CMs after injuries such as MI, zebrafish have been found to efficiently regenerate their heart upon injury. Although zebrafish have a simpler heart anatomy than that of humans, it serves as an important model for regenerative medicine because they have a similar histological composition when compared to other vertebrates. When looking at the heart specifically, the zebrafish was found to regenerate about 20% of the ventricle after amputation (González-Rosa et al., 2017). In humans, injuries to the heart result in formation of scar tissue, but zebrafish have exhibited minimal scar tissue formation during cardiac repair. This phenomenon occurs because zebrafish have the ability to activate stem cells or dedifferentiate somatic cells into stem cells that can be specialized into CMs and replace the damaged myocardium (Hesse et al., 2017). The mechanisms that allow zebrafish to regenerate its heart so efficiently are still unknown; however, uncovering these mechanisms will allow researchers to translate them to induce human heart regeneration. Thus, such a solution would require more funding, time, and research before being implemented.

Gene therapy has been considered to treat HF. Recent advances in cardiovascular research has provided insight into molecular mechanisms responsible for advancing HF. Such insights have allowed researchers to use gene therapy by targeting the genetic establishment of the cardiomyocyte and targeting specific molecular entities that seem to influence the development of HF (Vinge et al., 2008). While only a few clinical trials involving the application of gene therapy towards the treatment of HF have been conducted, the results of the studies provide promising preliminary data that will guide future researchers in exploring more specific genes, molecules, and signaling pathways that are involved with HF.

The proposed candidate solutions will focus on cellular and tissue engineering to rehabilitate heart function before end-stage HF. Recent advances in regenerative medicine has allowed researchers to determine various treatments to regain cardiac function. Specifically, the solutions are centered on cell-based therapy, particularly using stem cells, 3D bioprinting, and scaffold-free tissue engineering. These solutions are not only being tested for cardiac rehabilitation, but also for other organs that have lost physiological function. The solutions aim to use the patient's own cells during the development of the treatment, thus mitigating the likelihood of immune rejection. Furthermore, the solutions intend to maintain the regained heart function after the therapy is applied.

## Explanation of candidate solutions

The subsequent sections deliver an explanation of the design of the three proposed solutions examined: cell-based engineering, 3D bioprinting, and scaffold-free tissue engineering. After providing descriptions regarding the design, further insight will be given into the invasiveness, materials/resources used, cost, and other non-technical measures.

### *Cell-Based Engineering*

Cell-based therapies, specifically stem cells, are optimal for treating patients because it is more personalized than current drugs aimed to treat a wide variety of patients. Thus, stem cells are more precise, effective, and reduces the need for immunosuppressive drugs. While there are various stem cells used in regenerative medicine, this solution proposes using human induced pluripotent stem cells (iPSCs) to rehabilitate injured hearts. These cells are able to proliferate limitlessly while containing the patient's own genetic information and can differentiate into all somatic cells, including CMs. This is done by taking tissue cells from the patient and reprogramming them using reprogramming factors to produce human iPSCs (Lin et al., 2019). Once the iPSCs are produced, they can be differentiated into CMs located at different areas of the heart, such as the ventricles and atria. Recent data has indicated that the generated CMs have displayed the same electrical activity as human CMs (Kishino et al., 2020). Furthermore, the CMs were able to regain muscularization with the subject's host tissue when gap junctions were formed between the engrafted cells and the host tissue (Kadota et al., 2020).

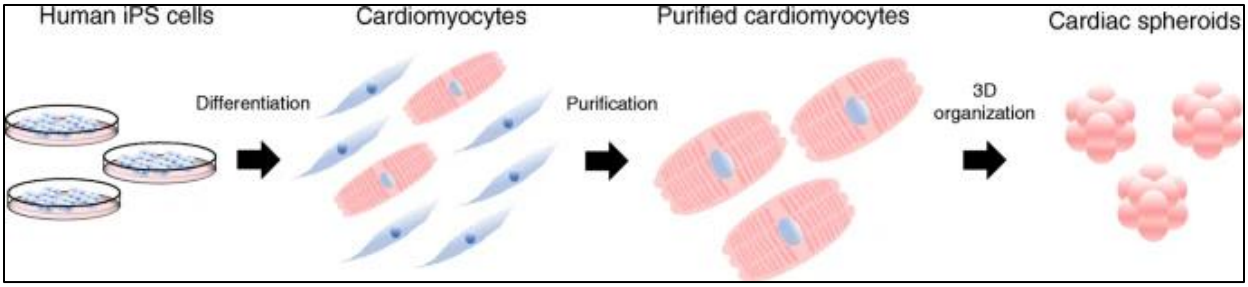


**Figure 5. Simplified schematic of developing CMs from human iPSCs**

Source: Kishino et al., 2020

Figure 5 displays a simplified illustration of engineering human iPSCs to differentiate into CMs that are later purified and used to develop a three-dimensional culture. While 3D cultures are useful for producing large amounts of CMs using equipment such as bioreactors or spinner flasks, the process is relatively costly because it necessitates the implementation of recombinant proteins to assist the cells into differentiating. This solution suggests using two-dimensional cultures because they can be more cost-effective since it uses low-cost reagents for differentiation methods using low-molecular-weight compounds and inhibitors to develop CMs. It is important to note that methods for both 3D and 2D cultures do not provide 100% efficiency when differentiating human IPSCs into CMs.

Once understanding how the CMs are derived using human iPSCs, the delivery method of the cells to the host heart must be taken into consideration. Currently, there are two different methods involved in delivering the human iPSCs into the host heart. One of the methods is epicardial attachment of engineered heart tissue (EHT). The other method is more direct, and it

utilizes am intra-muscular injection of the generated cells. Both methods have several associated advantages and disadvantages, but EHT has been shown to produce better retention of the induced CMs in the heart tissue of the host (Kadota et al., 2020). Table 1 provides a summary of recent trials using different PSC-CMs on swine and non-human primates (NHP) using both injection and attachment methods. Both human iPSCs and human embryonic stem cells (hESC)

**Table 1. Overview of using various PSC-CMs in large animal studies**

| Year | Animal | Cell | Cell number (*$10^6$) | Days after MI | Method | Duration (weeks) | Contractile function | Arrhythmia | Reference |
|---|---|---|---|---|---|---|---|---|---|
| **2012** | Swine | hiPSC-CM | 25 | 28 | Attachment | 8 | Improved | - | 46 |
| **2014** | NHP | hESC-CM | 1000 | 14 | Injection | 12 | No change | + | 40 |
| **2014** | Swine | hiPSC-CM | 6 | 7 | Injection | 4 | Improved | - | 42 |
| **2016** | NHP | NHP hiPSC-CM | 400 | 14 | Injection | 12 | Improved | + | 4 |
| **2018** | NHP | hESC-CM | 750 | 14 | Injection | 12 | Improved | + | 5 |
| **2018** | Swine | hiPSC-CM | 16 | 0 | Attachment | 4 | Improved | - | 43 |
| **2019** | Swine | hESC-CM | 1000 | 21 | Injection | 4 | No change | + | 41 |
| **2019** | Swine | hiPSC-CM | 100 | 28 | Attachment | 8 | Improved | - | 6 |

Source: Kadota et al., 2020

were used. When focusing on the human iPSCs, the 2012, 2014, 2018, and 2019 studies have indicated that improvement in contractile function, as well as an absence of arrhythmia, occurred with both injection and attachment approaches. Because more studies have yielded positive outcomes using the attachment method, this solution will similarly propose using an attachment approach.

Next, the design and delivery method must be translated to manufacturing the human iPSCs for patients. Because this proposed solution is a personalized approach to medicine, manufacturing such a product at a large scale would not be feasible because it is labor-intensive, expensive, and time-consuming among other reasons (Lin et al. 2019). Thus, the solution will be aimed at a limited number of patients using large cGMP-compliant manufacturing sites. Essentially, somatic cells would be extracted from the patient in a clinical setting. These cells would either be transported to a local cGMP manufacture site or a research laboratory for production. Figure 6 details how the human iPSCs are derived from fibroblasts taken from the patient, which are then reprogrammed and cloned to generate a large number of iPSCs, which can then be differentiated into CMs.

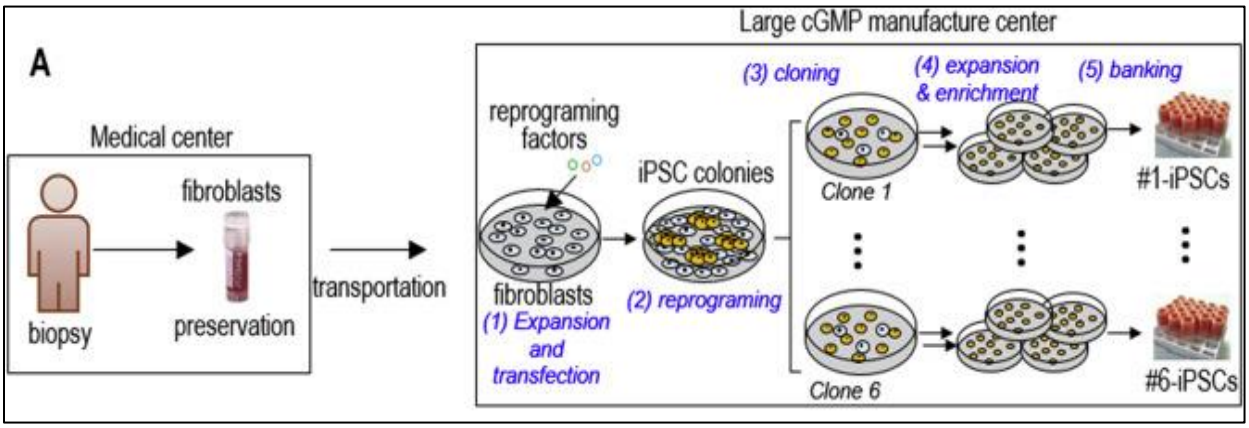


**Figure 6. Production of human iPSCs using cGMP manufacturing sites**

Source: Lin et al., 2019

There are various steps associated with the production of human iPSCs, and more to engineer them to differentiate into CMs, so well-trained lab technicians will be needed at both manufacturing and lab sites to manually produce a desirable yield of CMs. The process of producing sufficient amount of human iPSCs will take more than one month; differentiating them into CMs that are attached to the patients' heart tissue will likely take an additional two months. Since 2D cell cultures will be used throughout the process, there is a high risk of contamination with bacteria and cross-contamination with other patients' cells because such a system creates a relatively open environment for the cells. Thus, production of the cells must be carried out in high-grade clean room (at least class B level) for clinical purposes. Furthermore, once the cells have been transplanted, there are probabilities of poor engraftment; after transplantation, many of the CMs may not be able to survive, regardless of delivery method. In addition, immune rejection can occur because the process of producing the cells may yield in CMs that are not compatible with the patient's immune system. The process of transporting, preserving the cells, tracking the cells, etc. for each patient must also be considered. When all aspects of production are examined, the estimated cost to produce iPSCs would be about $50,000 (Lin et al., 2019), and differentiating them into CMs would bring more additional costs.

### *3D Bioprinting*

3D bioprinting, like cell-based therapies, will provide a more personalized approach to treating hearts after ischemic events leading to MI and consequently a loss of CMs and HF. By engineering cardiac tissue using the patient's own cells and images of their heart, 3D bioprinting aims to repair the damaged heart using scaffolds and/or bioinks that repair cardiac function. Various biomaterials used to create such engineered tissue include alginate, gelatin, collagen, as well as other applicable materials (Alonzo et al., 2019).

To being the process of constructing 3D bioprinted structures specific to the patient's needs, clinical imaging and/or CAD models are needed to produce accurate models of the patient's heart in its current state. To generate models that are anatomically precise, current imaging methods include: cardiac magnetic resonance (CMR), electrocardiography-gated computer tomography (CT), and volumetric 3D echocardiography (Alonzo et al., 2019). When comparing the three imaging techniques, volumetric 3D echocardiography can produce accurate images of cardiac structure, but it has limitations that could ultimately result in data loss with respect to the anatomy of the heart. CMR would be an optimal imaging technique when compared to both 3D echocardiography and CT scans because it can generate high-resolution images whole also retaining the ability to distinguish between tissue compositions within the heart.

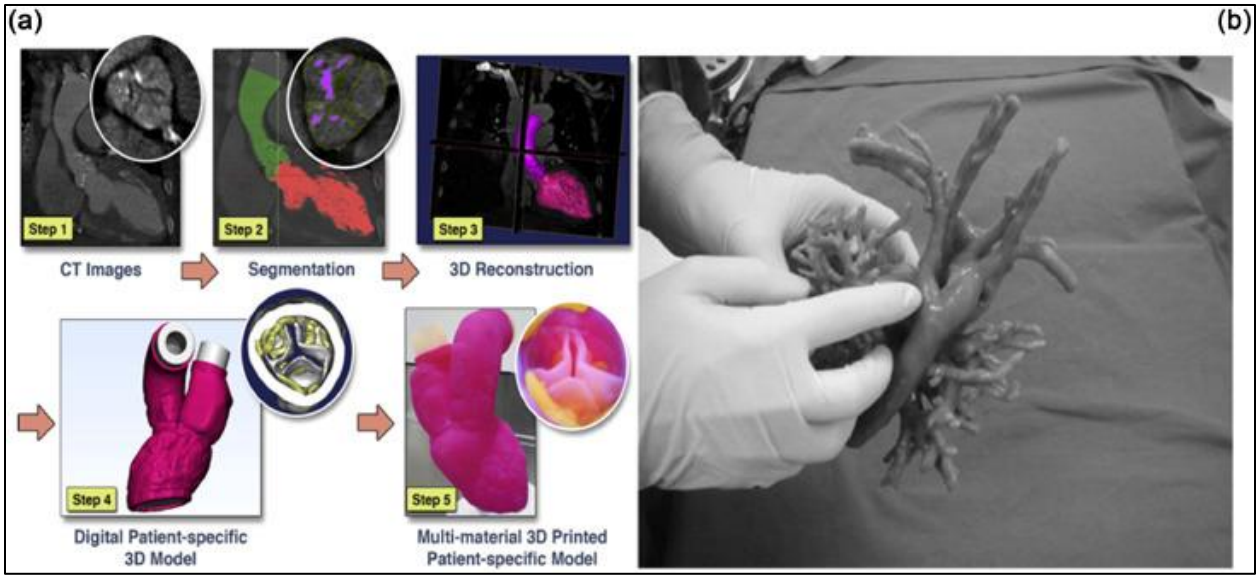


**Figure 7. Generating a 3D bioprinted model of patient heart using imaging techniques**

Source: Alonzo et al., 2019

After the images are obtained, a 3D digital model of the tissue is created using image segmentation, as seen in Figure 7. While this process generally uses CT scans, recent studies have indicated that CMR images can also be implemented in segmentation. Using a Digital Imaging and Communication in Medicine software (DICOM), which are generally open-source software, the developed image data sets from the scans will have anatomic geometry that is identified and segmented; the segmentation will depend on 2D image projections (axial, coronal, etc.). The software will stack the 2D images of the heart tissue and organize them so

that the pixels will generally accumulate in a defined intensity range so that they can be printed with one type of material. Finally, the model specific to the patient can be 3D rendered in a digital, Standard Tessellation Language (.stl) file using another computer aided software such as Slic3r, another open source software. Once the 3D model is created as an .stl file, even more adjustments can be made to the model so that it can closely remodel the tissue to specifically treat the patient's needs before being exported to a 3D bioprinter.

Once the files are ready to be 3D printed, the mode of bioprinting must be determined. Currently, there are several different types of 3D bioprinting, including: extrusion, inkjet, and stereolithography (SLA) bioprinting. Each type of bioprinting comes with its own set of advantages and disadvantages. For this project, stereolithography bioprinting would be the ideal bioprinting type.

**Table 2. Comparison of various bioprinting techniques**

| Bioprinting technique | Applications | Features |
|---|---|---|
| **Extrusion** | Creation of thick myocardial constructs; heart valve formation; construction of blood vessels | Viscoelastic bioinks; slow printing; medium cost; 3D; low resolution |
| **Inkjet** | Formation of blood vessels with HUVECS; 2D cardiac pseudo tissues | Low viscosity suspension; fast printing; low cost; 2D; high resolution |
| **Laser-assisted** | Blood vessel formation; cardiac disease modeling; creation of cardiac tissues | Hydrogel materials; medium speed; high cost; 3D; high resolution |
| **Stereolithography** | Creation of aortic valves; creation of implantable cardiac tissue with MSCs | Light-sensitive polymers; fast printing; low cost; 3D; high resolution |

Source: Alonzo et al., 2019

As seen in Table 2, the different types of printing techniques have been shown to produce different constructs of the heart, but each technique varies in terms of resolution and cost. Resolution is critical for producing a 3D model/scaffold that closely matches the construction of the patient's own heart. Thus, SLA printers look promising to accomplish this task. The SLA bioprinting technique utilizes a moving platform to allow the printing of subsequent layers. Furthermore, it uses lasers to create accurate microstructures within the 3D structure by photocuring the liquid polymers; essentially, UV, IR, or visible light laser beams are directed on the patterns, which are already designed in the bioprinting software, cross-linking the liquid polymer. After being exposed to the beam, the liquid polymers are traced over the pattern and are joined layer-by-layer to produce the final 3D structure (Alonzo et al., 2019). Using SLA

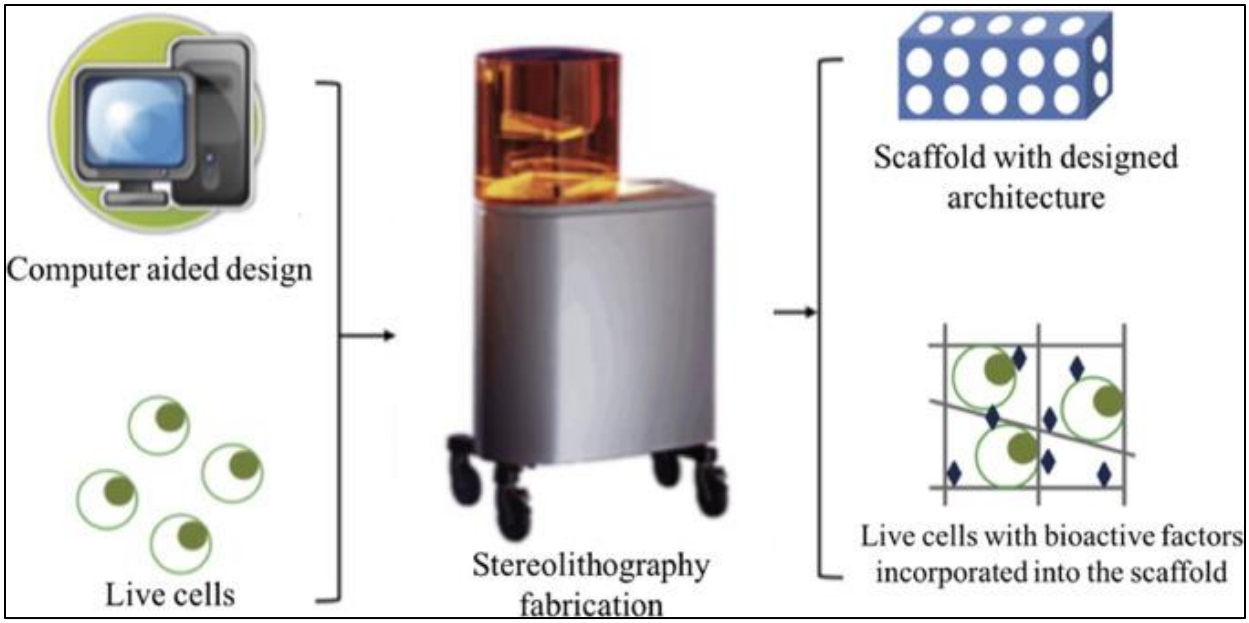


**Figure 8. Using SLA bioprinting to generate scaffolds containing patient cells**

Source: Alonzo et al., 2019

bioprinting methods have been used to create heart valve scaffolds successfully for over a decade, and research suggests that the printing method is suitable for printing cardiac tissue; SLA printers have been able to print 200 µm resolution prints in as little as 30 minutes, increasing efficiency. In addition to bioprinting the scaffolds, using stem cells integrated into the scaffold as seen in Figure 8 allows the resulting implanted tissue to improve cardiac function. This is because the SLA printer created multiple microchanneled hydrogel patches that contain the stem cells, specifically human iPSCs, with a controlled diameter of 500 µm, which intakes the cells' secreted growth factors and allowing it to spread throughout the engineered tissue. Using micro-SLA techniques to reduce the diameter to about 200 µm would allow for more precise microchannels, resulting in better distribution of growth factors.

Once the 3D structure is printed, postfabrication methods must be utilized to allow the material with incorporated cells/growth factors to adjust to its desired function. Because this proposal is focused on improving contractile function after MI to prevent end-stage HF, electrical and perfusion postfabrication methods will be used. Perfusion mainly focuses on promoting oxygen diffusion, cell attachment, and seeding cells deep within a scaffold by delivering essential nutrients throughout the scaffold. When implemented, perfusion improves cell viability and proliferation because it distributes the cardiac cells evenly in the engineered scaffold. In addition to perfusion cell seeding, electrical conditioning is needed to commence muscle contraction; electrical pulses on the bioprinted tissue revealed that cardiac cells were able to produce a contraction rate that is similar to pacemaker cells. This is particularly useful because CMs must coordinate their contractions, and electrical conditioning will obtain a simultaneous contraction of the CMs within the tissue (Alonzo et al., 2019).

When considering the costs associated with 3D bioprinting, one must first find a suitable SLA bioprinter. Costs for commercial bioprinters range from $10,000 to $200,000 depending on desired resolution and other features but most are within the range of $10,000 and $40,000. Obtaining applicable bioinks also present another cost; while there are various standard bioinks,

3 mL of cartilage bioink can cost about $199. Sourcing the stem cells from the patient will also increase the price of the solution. To decrease risks such as contamination, small cGMP manufacturing sites containing the bioprinters, incubators, bioinks, etc. will be needed, as well as high-grade clean rooms. Because sourcing the cells and manufacturing them at a large scale is similar to the cell-based therapy solution, the costs would be estimated to be similar ($50,000).

### *Scaffold-Free Tissue Engineering*

Engineering scaffold-free cardiac tissues is a proposed solution that provides a bridge between the differences of cell-based engineering and 3D bioprinting, which produces scaffolds, to promote CM growth. This solution offers less invasive delivery methods while simultaneously displaying the same augmented organization of scaffold-based CMTs without outsourcing biomaterials. Applying microengineering technologies allows technicians to accurately control the cellular organization, architecture, and geometry of the cardiac tissues (Patino-Guerrero et al., 2020). The resulting tissues are only comprised of the cells and their secretions.

Rather than using human iPSCs as the previously discussed solutions, the SF-CMTs will use human embryonic stem cells (hESCs) to produce CMs for the patient; these cells have been shown to successfully seed scaffolds in scaffold-based tissue engineering, so they will likely display similar results for scaffold-free tissues. Because there is a significant loss of CMs after MI, the design of the solution aims to create tissue to treat the injured area of the heart. However, because there is a significant loss of CMs, previous tissue engineering methods would need to improvise to create suitable macroscopic tissues. Thus, a different method to produce scaffold-free tissue patches containing hESCs to create macroscopic tissues would be needed.

The proposed method to implement the solution would first need to culture undifferentiated hESCs and then differentiate them into CMs. Previous studies suggested that the differentiated cells (contained in well plates) be placed on a rotating orbital shaker in an incubator at 37°C. The orbital shaker would rotate the cells at 40 rpm with an orbital radius of 1.9 cm (Stevens et al., 2011), resulting in the cells collecting in the center of each well and forming tissue in disk-shaped patches. Different cell concentrations can allow researchers to control the resulting cardiac patch size, enabling a personalized treatment for patients. Figure 9 displays how the cardiac tissue patches are formed after the CMs derived from hESCs are placed in an orbital shaker. The resulting patches ranged from 300 to 600 µm in terms of thickness, and the patches overall had areas where some were thicker than others. Furthermore, the CMs constituting the patches were able to contract in coordination with each other.

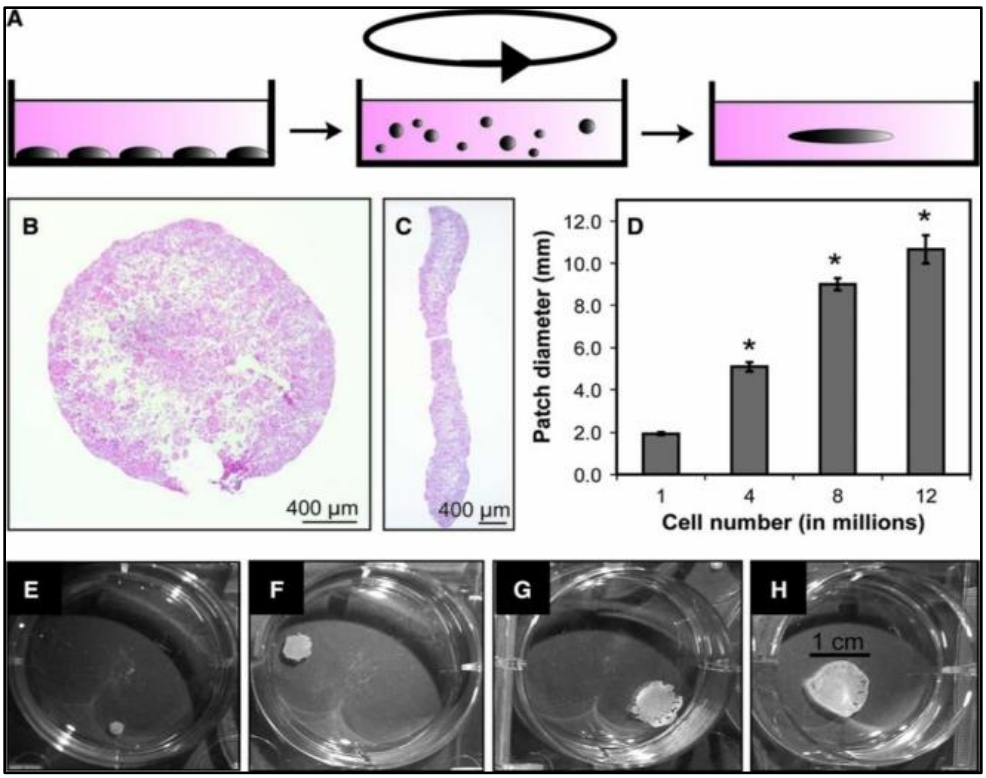


**Figure 9. Generating scaffold-free cardiac patches using hESC derived CMs and orbital shaker**

Source: Stevens et al., 2011

As seen in Figure 9, the results of the experiment that researchers can control the size of the patch based on the cell number determined from the hESC derived CMs step of the solution, allowing for the production of patient-specific cardiac patches.

As the hESCs differentiate into CMs, there is also potential for them to differentiate into other cell types, such as epithelial and endoderm cell elements among others of the three germ layers. These contaminating cells were identified in the resulting cardiac patches and tracked over time to determine their cellular activity. Experimental results shown in Figure 10 indicate that initially there were relatively high populations of each contaminating cell type. However, by day 11 there was either a significantly low population or were almost entirely eradicated from the cardiac tissue patches. In contrast, the number of CMs were able to considerably increase over time as the contaminating cells depleted. These results indicate that the cardiac patches have relatively strong resistances to contaminating elements, which would be ideal for patients during implantation.

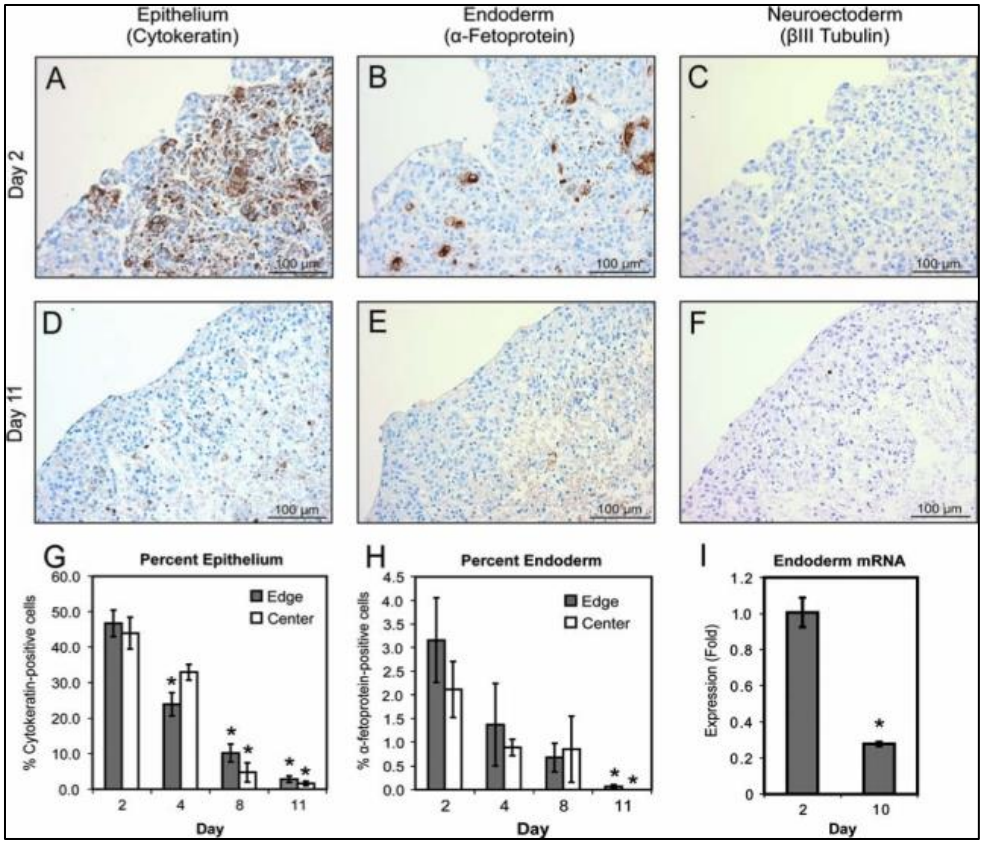


**Figure 10. Depletion of contaminating cell types over time**

Source: Stevens et al., 2011

The proposed method to implement scaffold-free tissue engineering for cardiac tissue differs considerably from most scaffold-based tissues, namely because it does not require the use of outsourced biomaterials and their own associated reagents. As a result, this solution can eliminate risks associated with scaffold-based tissue engineering. Only lab/cGMP manufacturing sites containing relevant tissue culture equipment and rotating orbital shakers in high-grade clean rooms are needed for large-scale production of these cardiac patches. Furthermore, orbital shakers can contain multiple well plates containing cells which increases efficiency and scalability of delivering the resulting cardiac patches to patients. The cost of rotating orbital shaker will at most reach $1000. The production of hESC derived CMs would result in most of the costs because skilled lab technicians, incubators, etc. are needed to produce them in a clinical grade manner. Thus, associated costs would be close to $40,000 (Stevens et al. 2011). Multiple shakers and incubators within a lab/manufacturing site could reduce delivery of the patches to about one month.

## Comparative assessment of candidate solutions

The table below seeks to summarize the different technical and non-technical aspects of the design and implementation of the proposed solutions.

**Table 3. Comparison chart for the three candidate solutions**

| | Cell-Based Engineering | 3D Bioprinting | Scaffold-Free Tissue Engineering |
|---|---|---|---|
| **Invasiveness** | Minimal | Moderate | Minimal |
| **Associated risks** | Contamination, human error, poor engraftment on target area/engraftment on non-targeted sites, immune rejection | Contamination, systematic errors associated with printers, immune rejection, toxic degradation products | Contamination, human error, non-homogeneous tissue patches |
| **Materials/resources needed** | Reagents, transportation, skilled lab technicians, clean rooms, iPSCs | Reagents, bioinks/biomaterials, SLA printer, clean rooms, iPSCs, skilled lab technicians, bioprinter technicians | Reagents, skilled lab technicians, clean rooms, hESCs, rotating orbital shaker |
| **Method of delivery** | Cells are directly implanted onto the injured heart | Printed scaffold-based tissues are implanted onto the injured heart after resecting the damaged area | Scaffold-free tissues are implanted onto the injured heart after resecting the damaged area |
| **Cost (for one patient)** | >$50,000 | ≈$90,000 | ≈$50,000 |
| **Setting** | Manufacturing site and/lab setting | Manufacturing site | Manufacturing site/lab setting |
| **Contractile function** | Low improvement | Moderate improvement | Moderately high improvement |

## Project Recommendations

The following section discusses the proposed solution to treat HF upon MI to circumvent significantly invasive surgeries. In addition, design and implementation challenges will be considered to examine the proposed solution's effects on patients, hospitals, and other organizations.

### Proposed solution

The three solutions discussed and summarized in the comparison chart (Table 3) all have associated advantages and disadvantages when attempting to rehabilitate cardiac function in patients that have suffered from MI and subsequently HF. Cell-based engineering therapies provide promising therapeutic effects with minimal invasiveness; however, the process of obtaining the iPSCs, culturing/differentiating them, and implanting them onto the injured heart contains too many risk factors that prevent it from truly improving cardiac function, and the resulting costs would not make it a viable solution. 3D bioprinting provides promising results in terms of improving cardiac function using SLA printers to print scaffolds containing stem cells. While the printing of the tissues is automated, technicians will still be needed to maintain the printers and ensuring that the printers are properly calibrated and cleaned between each use. Furthermore, 3D printing requires the use of outsourced bioinks and biomaterials, and when implanted into the patient's heart, they can produce inflammatory responses from the patient's immune system.

Thus, based on the aspects considered in Table 3, **scaffold-based tissue engineering** would be the optimal solution for treating HF. Specifically, this solution allows technicians to provide clinical-grade, patient-specific cardiac tissue patches to patients without introducing exogenous biomaterials. Instead of using iPSCs, this solution uses hESCs, which have been shown to successfully differentiate into CMs and perform in conjunction with the host myocardium after implantation. Thus, renewed cardiac function is likely to be retained after implantation which could produce similar, if not greater, survival rates than current treatment options. Furthermore, protocols can be improvised to increase the generated quantities of hESC derived CMs, which would allow physicians to treat more patients.

The produced cardiac tissue patches have been shown to retain cell viability and growth. Furthermore, contaminating cell elements within the patch have decreased in number as the patch grew, which would decrease the likelihood of immune rejection from the patient. The implanted tissue would directly replace the resected damaged tissue, which addresses concerns about treating the target site rather than inadvertently reaching unwanted sites.

Heart failure is a chronic, progressive illness that, to date, has no cure. However, HF can be remedied by focusing on providing engineering solutions at the root of the problem. Because scaffold-free tissue engineering is specific towards patient needs, a variety of patients could be treated without having to wait for a donor heart, resulting in a longer and better quality of life.

## Design and implementation challenges

Scaffold-free tissue engineering to produce patient-specific cardiac tissue patches, although promising, still has various difficulties associated with the implementation of its design. The patches require the use of skilled lab technicians in a lab or manufacturing setting. These technicians will mainly be responsible for culturing the hESCs and carefully differentiating them into CMs. As a result, human error is a foreseeable issue associated with the solution. Furthermore, the resulting cardiac patches are not always homogeneous; at times, the center of the patch can vary in thickness when compared to the edges of the patch. This is an implementation challenge because lab technicians need to cater to each patients' needs, and every injury is different from one another because it can be on the heart in several areas. Thus, the lab technicians will need to do further experiments utilizing different reagents to produce uniform patches that are specifically designed to treat the intended patient's heart.

In addition, creating cell culture systems in a clinical grade clean room within a manufacturing site and/or lab must take care to ensure that equipment and overall environment are sterile to limit possibilities of contamination. Technicians would also be needed to maintain the equipment itself and making sure that they are properly calibrated or ventilated. Because only a small sample is needed from the patient to be transported to a site, the sites do not necessarily have to be close to hospitals; however, placing sites closer to hospitals for the samples and the resulting cardiac tissues being delivered to the patient for implantation can reduce travel time and consequently any chances of contamination during delivery. Producing the patches on a large scale will also present implementation challenges because manufacturing sites will need to keep track of every well plate containing patient cells and make certain that they are properly differentiated, cultured, etc. before the final product is implanted.

Before being used in clinical settings, the patches and the processes to produce them must obtain FDA approval, and ensure that they are being compliant with EPA and OHSA guidelines. Upon implantation of the patch, cardiac surgeons will still need to perform surgery to implant the cardiac tissue patch on the injured heart. While this is not nearly as invasive as removing the patient's heart and replacing it with a donor heart, there are still risks associated with these surgeries, such as infection.

## Anticipated project outcomes and impacts

If implemented successfully, this solution would have a vast impact on a considerable amount of patients; in the United States alone, there are about 6.2 million adults that suffer from heart failure, resulting in $30.7 billion from healthcare costs, medicines, etc. (CDC.org, n.d.). Providing scaffold-free cardiac tissue patches provides an option for this population rather than pursuing invasive treatments such as LVADs and heart transplants. Moreover, the proposed treatment is personalized depending on the patient's specific heart injury, which would increase survival rates and quality of life.

Ultimately, this project would increase accessibility for a variety of HF patients in the U.S. Currently, the cost of LVAD implantations is about $170,000 (Shreibati et al., 2017), and the cost of heart transplants can cost about $1 million, including preparation and post-operation procedure costs (Bentley & Ortner, 2020). The proposed solution would significantly reduce costs to treat HF, thus allowing more patients to access this treatment. Furthermore, government

healthcare programs (Medicare), can reduce their spending on expensive procedures for HF and allocate funds that are needed elsewhere. Not only can the government benefit from this project, but private biotechnology companies, hospitals, and other clinical settings can as well; opening the market to this solution can provide numerous jobs for bioengineers, data scientists, lab technicians, etc. which would promote economic growth within the healthcare industry. This solution potentially be applied for other diseases, such as kidney and liver failure, increasing the potential for businesses and hospitals to treat a wider range of patients. Thus, scaffold-based tissue engineering has wide-reaching applications, providing low-cost, personalized care for patients while simultaneously providing job and business opportunities for healthcare industries and the government.

## Glossary

| | |
|---|---|
| **CM** | Abbreviation for cardiomyocyte.[1] |
| **AHA** | Abbreviation for the American Heart Association.[1] |
| **Heart failure** | Chronic cardiovascular condition that results in the heart's inability to properly pump blood to sufficiently supply the body with oxygen and nutrients.[2] |
| **hESC** | Abbreviation for human embryonic stem cells.[1] |
| **Hypertrophy** | Occurs after loss of organ cells, resulting in enlargement of the organ from remaining cells increasing in size.[3] |
| **Immunosuppression** | Occurs when the immune system is unable to fight infections. Can be induced by using medications.[4] |
| **iPSC** | Abbreviation for induced pluripotent stem cell[5] |
| **MI** | Abbreviation for myocardial infarction.[1] |
| **Myocardium** | The muscle layers of the heart wall, composed of cardiomyocytes.[6] |
| **Reagents** | Refers to the use of cell media, fetal bovine serum, and other related solutions needed for cell culture.[1] |
| **Scaffold** | A 3D biocompatible model that forms the basis for tissue formation.[7] |
| **Stem Cell** | An undifferentiated cell capable of specializing into any of the body's cell types.[3] |
| **Stereolithography** | A 3D printing technique that utilizes UV light and/or other laser beams to cross-link materials.[8] |
| **3D Bioprinting** | Utilizes biocompatible materials and inks to produce scaffolds encapsulated with cells.[9] |

[1] Author
[2] heart.org
[3] Hesse et al., 2017
[4] medicinenet.com
[5] Lin et al., 2019
[6] Tran et al., 2020
[7] O'Brien, 2011
[8] Alonzo et al., 2019
[9] Derakhshanfar et al., 2018

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