THE APPLICATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS TO HEAL TISSUES AFFECTED BY MYOCARDIAL INFARCTION

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Abstract *— Our paper will investigate the application of human induced pluripotent stem cells (hiPSCs) using a heart patch to heal tissue affected by myocardial infarction (MI). MIs, also known as heart attacks, affect upwards of 750,000 people in the United States. After experiencing MI, heart tissue becomes damaged and scarred, causing poor signal transmission and low pliability. Leaving the damaged heart untreated can lead to future diseases, including arrhythmia, heart rupture, and heart failure, since the heart does not regenerate muscle. Researchers at both the University of Alabama at Birmingham (UAB) and Duke University (Duke) have created human cardiac muscle patches (hCMPs) which are placed over dead heart muscle, providing more strength for contractions and a smooth path for electrical signals. In addition, the patch secretes enzymes and growth factors to aid in the recovery of damaged tissue. These researchers developed an ingenious process utilizing an oscillating platform and hiPSCs to create the patch. Since stem cells come from the patient, there is an extremely low likelihood that the body will reject the treatment; thus, the treatment will allow the heart and patient to function at near full potential. This paper will discuss current treatments for MI, the development and use of the heart patch, and possible ethical concerns raised regarding the patch.*

Key Words — Heart attack, Medicine, Product, Stem cell, Tissue engineering.

HUMAN CARDIAC MUSCLE PATCHES AND MYOCARDIAL INFARCTION

Heart attacks, or myocardial infarctions (MIs), affect upwards of 750,000 Americans annually [1]. Fifteen percent of people among them will die from their complication [2]. The Center for Disease Control states: "1 in 3 people will die from all types of cardiovascular disease, roughly the same number people in the United States each year as cancer, lower respiratory diseases, and accidents combined" [3]. These heart disease statistics will only increase in the years to come, as obesity, diabetes, and other poor health choices increase the chance of heart attacks across all ages [2, 4]. Suffering from a heart attack hinders one's ability to be physically active and usually leads to fatigue and depression in the victim [3].

Hospitals and taxpayers both suffer a major blow because of the volume of heart disease cases in the United States. 12.4 million people visit primary care physicians because of heart disease annually, and $313 billion are devoted to heart disease related costs every year [2]. Families who experience heart disease must deal with not only medical bills, but lost wages and a decreased standard of living [3]. What could possibly cause this much of a burden on modern Americans?

Medical Background

MIs are generally caused by plaque buildup in the coronary arteries, large blood vessels that carry oxygenated blood to the heart muscle [5]. The National Heart, Lung, and Blood Institute defines plaque as "fat, cholesterol, calcium, and other substances found in the blood. Over time, plaque hardens and narrows your arteries" [6]. When coronary arteries get blocked by plaque, the heart muscle lacks enough oxygen to pump. An oxygen deficit severe and long enough can trigger an MI, which leads to sudden death of a large portion of the heart muscle [5]. Dead cells scar, lose cell-to-cell communication, and cannot contract to pump blood. Arrhythmia, or irregular heartbeats, and heart failure can occur if the scar tissue is left untreated.

To treat heart disease, current permanent solutions are quite expensive and difficult to perform, while most short-term solutions are only administered immediately after much of the damage has already been done [7]. Surgery is usually the most viable option for patients with heart complications but comes with high risk and financial burden.

CURRENT SOLUTIONS

The patient can be treated with drugs, surgery, or both depending on the cause of the MI. While drugs do not cure the disease, they relieve the persistent symptoms and stop further deterioration in the short-term. If it is not enough, surgery may be required. Surgery provides a reliable and resilient solution to complications related to heart disease but does not reverse the patient's underlying heart problems. Surgeries are also costly and require extremely skilled professionals to perform the operations, resulting in long waiting and planning periods

before receiving critically needed care. Below, we discuss the four most prevalent solutions: daily medication and lifestyle changes, bypass surgery, heart transplant, and minimally invasive heart surgery.

Daily Medication and Rehabilitation

Daily medication and rehabilitation fix the root problem of heart disease: plaque buildup in the main arteries of the heart. However, they do not reverse the problems that the dead heart tissue creates. This method of treatment is difficult to apply on its own because having a damaged heart prevents the patient from exercising heavily or at all, which is key to helping lower cholesterol and fats in the body. Therefore, medication and dieting are prescribed after the patient undergoes any type of surgery.

For post-treatment, drugs such as diuretics, vasodilators, and statins are prescribed. Diuretics remove excess water and sodium in the body through urination to help lower blood pressure [8]. Furthermore, it decreases the buildup of fluid in the lungs and other parts of the body. Vasodilators relax blood vessels to increase blood and oxygen flow to the heart to reduce chest pain. Statins reduce the amount of harmful cholesterol in your body [8]. These drugs and many more working in conjunction can extend the patient's life for more than a decade.

Medications are prescribed based on a patient's risk of blood clots and bleeding. The type of medication and duration of treatment is determined in conjunction with the patient's healthcare provider [8]. Unfortunately, these drugs are expensive and must be taken for at least 12 months following the MI. A popular brand of statin called Lipitor can cost up to 200 dollars a month, depending on the dosage [9]. Patients are usually prescribed more than one drug, causing bills to accumulate rapidly. Patients also are required to have regular checkups with their doctors to document improvement in their health or mitigate unforeseen complications.

Heart Bypass Surgery

Heart bypass surgery, also known as coronary artery graft surgery, is carried out in cases where the patient has a diseased artery or a blockage that cannot be removed in any number of arteries [10]. Bypasses can be single, double, triple, or quadruple, depending on how many arteries are irreparable.

The procedure constructs a detour around a blocked artery to improve blood flow to the patient's heart. First, the patient is put under general anesthesia, causing them to feel no pain throughout their body [11]. A surgeon then proceeds to remove an artery from another part of your body, usually the legs, and uses it as a blood bypass around the blocked artery in your heart.

Patients have lessened or no chest pain, a lower chance of heart attack, and more energy. Although heart bypass surgery is common and generally successful, it carries many risks. Because it is an open-heart surgery, suffering from another heart attack is highly possible if the surgery is done improperly. Other risks include changes in heart rhythm, lung infection, nerve injuries, and death [11]. After the procedure is completed, the patient usually stays in the hospital for another week to make sure both the graft is functioning properly, and their vital signs are normal [10]. It still takes anywhere from six to twelve weeks to make a significant recovery given no other complications arise. Patients usually experience little to no symptoms for ten to fifteen years after the procedure. However, the patient is kept on daily medication and a strict diet following the procedure to mitigate and prevent future plaque buildup [10].

Heart Transplant

This operation is designed for patients with a failing and diseased heart in which their failing organ is replaced with a healthier donor heart. It is a treatment that is reserved for people who have tried medications or other surgeries but have seen no significant improvement in their health [12]. The patient's heart can become non-functioning in several ways. Frequently, it is because one or both ventricles are not functioning properly, and severe heart failure is very likely. Ventricular failure can happen in many forms of heart disease but is more common in defects with a single ventricle or if long-standing valve obstruction or leakage has led to irreversible heart failure.

Doctors remove the patient's heart by transecting the aorta, main pulmonary artery, the superior and inferior vena cavae, and dividing the left atrium [13]. As a result, this exposes the back wall of the left atrium with the pulmonary vein openings in place. The surgeon connects the donor heart by sewing together the recipient and donor vena cava, aorta, pulmonary artery and left atrium.

After the extensive surgery, patients are placed in the intensive care unit (ICU). Many are generally moved to a regular hospital room after a few days in the ICU and will usually remain in the hospital for a week or two [12]. The amount of time spent in the ICU and hospital can vary. Patients are closely monitored, after leaving the hospital, at an outpatient transplant center by their respective transplant team. Due to the frequency and intensity of the monitoring, many people stay close by the transplant center for the first three months. Afterward, the follow-up visits are less frequent, and it is more convenient to travel back and forth for follow-up visits. In addition to this, they are monitored for any signs or symptoms of rejection, such as shortness of breath, fever, fatigue, infrequent urination or weight gain. Patients have regular tests, including blood work, echocardiograms, electrocardiograms and heart biopsies [12].

The main risk associated with this procedure is rejection of the organ. If the donor heart is not compatible with the patient's body, the patient's immune system will target the donor heart and destroy it as if it were some sort of pathogen. Along with this risk comes similar risks to open heart surgery, including arrhythmia.

Minimally Invasive Heart Surgery

MIHS is a safer and better alternative to heart bypass surgery. Unfortunately, not everyone can undergo the procedure. The patient's doctor thoroughly reviews the patient's medical history and performs some physical tests to determine if this type of surgery is appropriate [14]. Potential benefits of MIHS include less blood loss and infection risk, a shorter hospital visit, and a reduced recovery time [14].

The type of MIHS we will discuss is robot-assisted MIHS. In lieu of cutting open the patient's chest to access the heart, surgeons make small incisions between the patient's right ribs for device insertion [14]. The surgeon utilizes a remote console that controls robotic arms that exactly replicates the movements he or she makes. The Mayo Clinic states: "The surgeon works at a remote console and views your heart in a magnified high-definition 3D view on a video monitor" [14]. A team of assistants replaces attachments on the robot arms as the procedure continues. Other than this, MIHS is essentially the same as heart bypass surgery, where an artery from one part of the patient's body gets grafted onto the heart.

MIHS carries the same risks as other open-heart surgeries, including chance of stroke, arrhythmia, and wound infection. The recovery period for MIHS is significantly shorter than that of bypass surgery, but the patient still cannot do many normal things for a short while after the surgery, like driving a car [14].

All the methods have a long timeframe and only cure either the underlying reason the patient's arteries are being clogged or the damage done to the heart because of the MI. A patient-specific, reliable, and cost-efficient replacement to the current methods is necessary to reduce constant strain on hospitals across the country that need to treat heart attack patients.

HEART MUSCLE PATCHES: AN EFFICIENT SOLUTION

Researchers at the University of Alabama Birmingham (UAB) and Duke University (Duke) have recently developed a tissue engineering alternative to current MI surgeries. They created the human cardiac muscle patch (hCMP), which can be placed over damaged heart tissue. The goal of their research was to create a patch suitable for human use (4 cm x 2 cm x 1.25 mm) that solved issues that dead heart muscle created. The hCMP significantly improved cell-to-cell communication and forcefulness of heartbeats, allowing swine heart affected by MI to function statistically significantly better than their untreated MI counterparts [15]. Stem cells and their broad uses have manifested yet again in a solution for MI damage. This patch holds promise to have widespread use in hospitals as an alternative to current operations. Below, we discuss how stem cells and tissue engineering techniques used in creating the hCMP came to be and why they work.

TISSUE ENGINEERING TECHNIQUES

To understand how the UAB and Duke researchers created the hCMP, one must first understand what human induced pluripotent stem cells (hiPSCs) and gel scaffolds are and how they provide the basis for creating the hCMP.

Human Induced Pluripotent Stem Cells

Genes encode proteins which decide a cell's function. Cells only express a small fraction of genes at any given time. Therefore, the expressed genes determine the behavior of that cell. Every cell has a unique set of transcription regulators that differentiate cells from one another [16]. These regulators function to either induce or suppress transcription. With the manipulation of these transcription factors comes the invention of pluripotent stem cells. By introducing four specific genes encoding for transcription factors, cells can be converted into pluripotent stem cells.

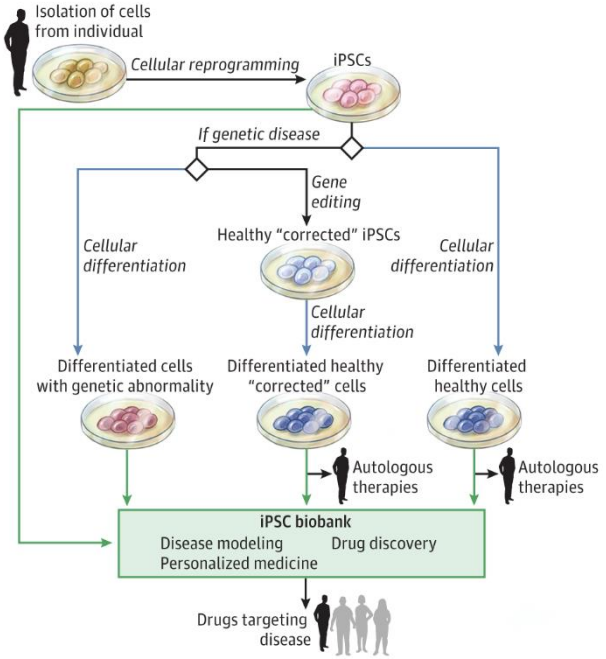

FIGURE 1 [18]
Flowchart of iPSC creation

hiPSCs are derived from human skin or blood cells that have been reprogrammed back into an embryonic-like pluripotent state that enables the development of an unlimited source of any type of human cell needed for therapeutic purposes [17]. These cells hold much promise in the field of regenerative medicine because they can multiply indefinitely and have applications in biology, drug development, and transplantation. hiPSCs are genetically forced to express genes and factors important for maintaining the defining properties of embryonic stem cells.

Since iPSCs can be derived directly from adult tissues, they not only bypass the need for embryos, but can be made in a patient-matched manner, which means that everyone could have their own pluripotent stem cell line. An unlimited supply of the patient's own cells could be used to generate transplants without the risk of immune rejection. While iPSC technology has not yet advanced to a stage where therapeutic transplants have been deemed safe, iPSCs are readily being used in personalized drug discovery efforts and understanding the patient-specific basis of disease.

Unlike iPSCs, hiPSCs still require much clearance before being used in clinical trials. Currently viruses are used to introduce the reprogramming factors into adult cells, and this process must be carefully controlled and tested before the technique can lead to useful treatment for humans. In animal studies, the virus used to introduce the stem cell factors sometimes causes cancers. Researchers are currently investigating non-viral delivery strategies. In any case, this breakthrough discovery has created a powerful new way to "de-differentiate" cells whose developmental fates had been previously assumed to be determined. In addition, tissues derived from iPSCs will be a nearly identical match to the cell donor and thus probably avoid rejection by the immune system.

Fibrin Gel Scaffolding

Gel scaffolds are a sort of skeleton scientists use to grow cells on. The type of scaffolds used by the UAB and Duke researchers was a fibrin hydrogel. Fibrinogen is a protein found in the body that aids in wound healing by providing a natural scaffold for other proteins and cells to bind to. Upon interacting with the chemical thrombin, fibrinogen splits into fibrin, which rapidly creates an extracellular-matrix-like environment near the wound. Fibrin holds many physical properties useful to research scientists. These include biodegradability, biocompatibility, easy purification, high abundance and modifiability, and high tensile strength upon stretching [21]. Fibrin is quite soft compared to other biopolymers, says Janmey, Winer, and Weisel, which lends itself well to being a mold for cells to grow into [21].

Fibrin scaffolds are created in a similar fashion to how they are created in the body. Fibrinogen, found abundantly in blood plasma, is fully dissolved in a buffered saline solution. The solution then undergoes dialysis, a liquid purification process, for twelve hours. The dialyzed solution is filtered twice more before being transferred into small dish called a 24 well plate. In each well or cavity, fibrinogen is combined with thrombin and calcium chloride to trigger the polymerization reaction that turns fibrinogen into fibrin, creating the scaffold. The plate is incubated for an hour at 37° C to fully set the scaffold [21]. This can then be used as a site to grow any type of cell, usually stem cells, given the correct cell growth media.

Since fibrinogen is readily available in the body, the cost of creating fibrin scaffolds is low. This also means that the body will not mistake the compound for a pathogen and attempt to destroy or get rid of it. As the stem cells begin to integrate into the heart, the fibrin scaffold also gets broken down and recycled. These reasons are why fibrin scaffolds are a very sustainable foundation to grow hCMPs on.

PATCH PRODUCTION

The goal of the UAB and Duke research was to create a clinically-relevant muscle patch to improve heart function following an MI. They achieved this by combining a few key principles: cells grown in 3D environments function better than those grown in 2D environments, hiPSCs differentiated into cardiomyocytes are more efficient and less likely to die when grown with endothelial cells and smooth muscle cells, and a rocking culture process helps cells mature faster [15].

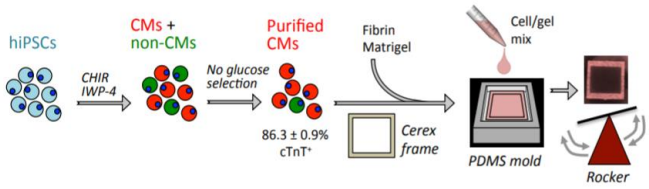

FIGURE 2 [15]
Flowchart of the hCMP culture process

Each patch is a mixture of three cell types: 4 million cardiomyocytes, or heart-muscle cells; 2 million endothelial cells, which are well-known to help cardiomyocytes survive and function in a micro-environment; and 2 million smooth muscle cells, which line blood vessels [21]. This ratio of 2:1:1 provides the optimal support for the cardiomyocytes and maximizes functionality. Figure 2 above depicts the process that the researchers used to grow the muscle patches. The cell mixture was combined with fibrinogen and placed on a plate with thrombin to start the scaffold polymerization reaction [15]. Submerged in growth solution, the patch was placed on a dynamic rocker to simulate the environment it would eventually be applied to.

After just one day of culturing, the cells began to send signals to each other and contracted like a cohesive muscle. A minimal number of cells died and the same 2:1:1 ratio was maintained after a week of growth [15]. The patch also contracted stronger when it was stretched compared to when it was unstretched, which is how cardiac muscle is usually is. Compared to patches created on a 2D scaffold and those created without endothelial or smooth muscle cells, the hCMP displayed better electrical signaling and muscle contraction properties.

Results

The researchers used a swine model to test their newly developed hCMPs. They used four groups for testing: MI+hCMP (treated with two hCMPs), MI+OP (treated with

cell-less fibrin scaffolds), MI (untreated), and Sham. In all MI groups, an MI was induced by blocking a major coronary artery for 1 hour [15]. The Sham group was operated on as if they experienced an MI, but in fact had not [15].

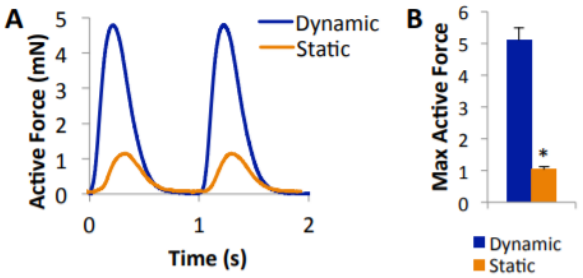

FIGURE 3 [22]
Graphs of A) force of beating hCMPs and of B) max force of a hCMP beat

Magnetic resonance imaging was used to determine how the hearts were functioning four weeks after experiencing the MI [15]. Various properties of the left ventricle, the chamber of the heart that pumps oxygenated blood to the entire body, were investigated. The size of the damaged area and thickness of the left ventricle were both reduced in the MI+hCMP group, signifying a better healing process taking place as compared to the control and untreated groups [15]. The hCMPs also did not create arrhythmia in any hearts, unlike what may happen after a surgery like heart bypass. In Figure 3, graph (A), the significant difference between hCMPs cultured dynamically and statically can be seen by the force measured every time the patch beat. Graph (B) shows that the dynamic cultured muscle patches contracted much more forcefully than patches cultured statically, suggesting that the dynamic culture process helped the cells grow to function like a real heart.

Benefits and Drawbacks

The implications of the results obtained from this research are massive. The main problem with damaged heart tissue is that the dead tissue never shrinks in size or gets replaced. The hCMP functions as a cellular bridge that spans the dead muscle gap, allowing normal cellular signaling and contraction for blood pumping. The hCMP also helps reduce the amount of cell death and size of the damaged tissue. On top of an eleven percent stem cell survival rate, which is relatively high, hCMPs would be extremely effective if they could be used in hospitals [15]. If implemented, hCMPs would be a cost-effective and reliable replacement for heart transplant surgeries which are expensive and need a biocompatible heart donor.

Part of the beneficial effects of the patches may come from the release of tiny blebs called exosomes from cells in the patches. These exosomes, which carry proteins and RNA from one cell to another, are a common cell-to-cell signaling method that is incompletely understood. In tissue culture experiments, the researchers found that exosomes released from the large heart-muscle patches appeared to protect the survival of heart-muscle cells [15].

hCMP technology is a double-edged sword, however. Currently, open heart surgery would be required to graft patches onto the patient's heart. Medication and rehabilitation would also still be required for patients to recover fully. The hCMPs only solve the problem of dead heart muscle, not the problem of plaque buildup in the arteries.

OTHER APPLICATIONS OF PATCH TECHNOLOGY

hCMP technology is easily extendable to other areas of treatment. Although its novelty and main usefulness comes from the dynamic culture process and its application in MI treatment, the same cell and scaffolding methods can be used to create other tissue patches to treat other ailments.

For example, third degree burns damage much of the lower layers of the skin, damaging nerves and heavily scarring the tissue there. Treating the burn site with a specially grown muscle patch could restore muscle control and cell signaling to the area and possibly reduce scarring in the long term. If hiPSCs were differentiated into nerve tissue instead, paralysis victims could have nerve patches grafted onto areas that have lost nerve connection. A more direct application could also be reversing scarring in the liver or kidney due to poor health habits by grafting organ-specific patches onto damaged areas. Finally, with a different type of scaffold, large blocks of muscle could be grown to help patients suffering from muscular dystrophy.

By changing both the cell type and scaffold system, the creation of artificial organs may be feasible using the dynamic culture process. Regulated and guided polymerization of fibrin could create structures shaped the same way as various organs, and relevant hiPSCs could be dispersed throughout. Submerging the system in appropriate cell growth media and dynamically culturing for long enough could yield a functioning artificial organ.

A DISCUSSION OF ETHICS

As with most topics regarding human biology, misguided applications of this technology pose serious ethical issues. A pertinent problem that could arise with this technology could be mass production of artificial organs and other tissues to delay the aging process. Since fibrinogen is easily controlled and can be triggered very specifically to split upon contact with thrombin, the future may hold the creation of complex organ scaffolds instead of simple prism-like ones. If organ scaffolds were introduced to the market, prices would be high to begin with, no doubt. Not everyone would have access to this life-saving product because of socioeconomic or geographic barriers. However, as science progresses, more people will find better ways to make cheaper scaffolds. If the market

becomes saturated with readily available organs, there is a thin line drawn between using organs to cure diseases and using organs to enhance the body. Another issue might arise if new genes are discovered that allow cells to secrete performance-enhancing substances. Would it be legal for an athlete who needed a new heart to get this special replacement?

Physical enhancements created with this technology should not be bought and sold, because they only exist to widen the gap between the rich and the poor. It would be highly unethical to use hCMP technology or its derivatives for pure enhancement purposes, but where disabilities and ailments exist, hCMP technology should be used to better and extend the patient's life.

At first, this treatment will probably be reserved for the best quality hospitals. As the hCMP procedure becomes cheaper and more sustainable, more hospitals will be able to adopt this technology. When treatments are easily accessible and relatively inexpensive, society benefits, and the treatment is sustainable. In the coming years if hCMPs are allowed into clinical trials, researchers must strive to make the process of creating them more efficient so that this objectively better treatment to bypass and transplant surgery can be sustainable.

REPLACING CURRENT SOLUTIONS WITH THE hCMPs

Heart disease places a major burden on healthcare systems today. Heart tissue and muscle damaged by MIs not only do not function as efficiently as before but cause later heart complications. Current solutions either temporarily reduce symptoms or cover up underlying problems. The hCMP developed by researchers at UAB and Duke seeks to uproot current methods of treating MIs by fixing one of the two main problems: dead heart tissue preventing the heart from pumping blood properly. Although medication and rehabilitation are needed to treat the other main problem, fat and cholesterol buildup in the body, hCMPs seem to be much better solutions to MIs than current surgery techniques.

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