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Induced Pluripotent Stem cells and their treatment for Cardiac Disease

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_____

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Jay Small served as the primary author of the Background and coauthor with Alberto Phillips for the induced pluripotent stem cells sections of the results and Discussion sections. He also helped in the revision process of the project.

Alberto Phillips

Alberto helped write the background sections of the report as well as results and discussions sections. He aided in the editing of the methodology sections and was primary author of the conclusions section of the report.

James Ventola

James Ventola was primarily responsible for writing the market analysis portion of the project. He assisted in writing all portions of the project relating to the business aspect of the project and conducted the break even points, return on investment and pricing strategies, as well as target markets, industry update and insurance policies. He also wrote the methodology section of the report.

Abstract

With the widespread issue of cardiovascular disease, there is a call for the development of technology to help in the recovery of heart attack victims. The development iPSCs alleviates the damage experienced by myocardial infarctions. Current research suggests that iPSCs are a promising solution to the issue of cardiac repair, and can help regenerate healthy tissue in damaged areas. This study aims to explore these possibilities and apply them towards the analysis of full manufacture and sale of this technology.

Executive Summary

Cardiovascular disease has been one of the leading causes of death in the United States for over a century. Each year over $108.9 billion is spent to treat patients suffering from myocardial infarction. The current methods of heart repair are limited by a lack of technology and are challenged by the inability to restore function to dead tissue. Surgical options are considered the most effective treatment option at this point in time, yet limitations for these procedures still remain. These include rejection of allogeneic tissues, infection, and a limited number of organs just to name a few.

Stem cells have the potential to overcome these limitations and act as a more effective form of treatment for a myocardial infarction. A stem cell is classified as an undifferentiated, self-renewing cell that has the potential to differentiate into other types of cells. There are many different types of stem cells in the human body including embryonic, mesenchymal, cardiac, and induced pluripotent stem cells. In the United States there is still a lot of controversy surrounding embryonic stem cells even though techniques have been developed to obtain them without destroying the blastocyst. Nonetheless due to this stigma they were not analyzed as part of this paper.

Mesenchymal stem cells sometimes referred to as bone marrow stem cells, have shown the potential for treatment of a heart attack in limited clinical trials where they were found to improve ejection fraction. Some studies have shown that they may be limited by an inability to accurately target injury sites in vivo. Cardiac stem cells are newly discovered cells that were found in a specialized compartment in the heart. There is still a lot to be learned about these cells, but they are believed to be involved in homeostasis of the adult heart. They have shown promise for treatment in trials on myocardial infarctions in mice as well. The scarce quantities of

these cells in the human body coupled with the lack of research about them are their main restricting factors.

In 2012 Dr. Shinya Yamanaka and Dr. John B. Gurden were awarded the Nobel Prize in medicine for their discovery of induced pluripotent stem cells. These are stem cells that are converted from adult epithelial cells into “embryonic-like” stem cells via the overexpression of four cellular transcription factors: Oct3/4, Sox2, CMY-C, and KLF4. These cells have the most potential to become an effective medical treatment based on the fact that they can be induced from adult cells. There have also been studies showing that induction efficiency can be increased by using micro RNA to silence induction inhibitors. A very serious concern of this technology is that the induction process can stimulate an oncogenic response in cells. Scientists are currently investigating the use of alternative transcription factors to avoid this response.

A market analysis was conducted in order to understand how profitable these cells could potentially be. Many factors needed to be considered to determine this including the state of the present industry, the target market, pricing strategy, and potential strengths, weaknesses, opportunities, and threats to the technology.

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1. Background Chapter

Cardiovascular disease (CVD) has been the leading cause of death in men and women in the United States every year since 1900, except in 1918 (influenza was the leading cause this year), with coronary heart disease being the most common[1, 3]. An initial heart attack is experienced by 785,000 people every year, with 470,000 who have already had a heart attack experiencing another later in their life[1]. This puts a $108.9 billion dollar burden on the Unites States economy[1]. Ischemic heart failure occurs when the coronary arteries that supply oxygenated blood to the heart are blocked[2]. Blockage of the coronary arteries is mainly caused by plaque formation that prompts blood platelets to stick together forming a blood clot, or complete obstruction of the artery by the plaque[2]. Once the flow of oxygen to the heart is impeded, the heart tissue is deprived of oxygen, therefore initiating mass cell death[2].

Identifying a heart attack is crucial for survival. Chest pains are the most common symptom and may spread to the arms, shoulder, and jaw[2]. Although these symptoms are common, this does not mean that they will occur. Elderly people, people with diabetes, and women are more likely to experience a phenomenon known as a silent heart attack, where little to no chest pain is present[2]. There are several ways to test for heart damage in a patient. Electrocardiograms may be used to identify damaged areas or a troponin blood test can show if there is tissue damage[2]. After the tissue has been infarcted, the resulting damage can lead to scar formation, increased pressure, ventricular remodeling, and ultimately death if left untreated[3]. The restoration and regeneration of damaged tissue is therefore the most viable option for optimal heart repair[3]. Traditionally heart repair has been challenged by the lack of technology and methods needed to completely restore function. Current pharmaceutical treatments involve beta-

blockers, diuretics, and angiotensin-converting enzyme inhibitors[3]. Although these treatments may relieve the heart from the burden caused by tissue damage, they do not restore function to the dead cells[3]. Surgical options can vary from implanting a pacemaker to receiving complete organ transplantation[3]. Although the implantation of a mechanical device to assist the heart can provide a long-term solution, the fact remains that these devices are prone to infection, mechanical failure, and blood clots[3]. The optimal choice currently available for heart infarction victims is complete heart transplantation, although problems such as rejection and organ accessibility limit the practicality of this option[3].

A major concern with any new innovation in the healthcare field is cost and the level of coverage by insurance companies for new treatments. Stem Cells, being a rather new technology, are expected to be expensive when they come into the market.

For this project there were two main problems to be considered. The first issue was to find the best stem cell of embryonic quality to use in the regeneration and repair of heart tissue. These stem cells need to be easily applicable to patients and the issues of rejection, compatibility, and timing need to be resolved. The term "of embryonic quality" refers to a pluripotent, undifferentiated cell that can be used to treat infected or infarcted areas of the heart. It does not mean that actual embryonic stem cells are planned to be used. The use of embryonic stem cells as a treatment or remedy remains a very controversial topic in modern medicine. The second problem that was considered was the issue of profitability of this technology. This was determined by conducting a thorough market analysis of the stem cells including but not limited to creating a SWOT (Strengths, Weaknesses, Opportunities, Threats) analysis, studying the supply-chain management of the technology, looking at current trends in the marketplace, and determining successful marketing strategies. Also, the team will consider looking at the

ethical concerns around embryonic stem cells to see if those concerns carry over to other types of stem cells. Currently there is no industrialization process for viable mass production of these cells. To determine the profitability of the stem cell product, it is important to assess every stem cell from production to administration into the final patient.

Current Treatment for heart failure

Current methods for the treatment and prevention of heart attacks are being revised and improved upon, but mortality rates still remain high and treatment after an infarction remains complicated. It is estimated that in the US there are 1.1 million heart attack survivors every year[15]. To date, there are two approaches for the treatment of CVD: a pharmacological approach and surgical intervention[14]. The treatment of CVD through pharmacological therapy includes using beta-blockers, diuretics and angiotensin-converting enzyme (ACE) inhibitors. These drugs are even limited in their usage towards treating CVD. Patients have to meet certain criteria involving the extent of the damage, the location of the damage, and the stability of their circulation[16]. Furthermore, ACE inhibitors have to be supported with the intake of angiotensin II type 1 receptor blockers[17]. Drug metabolism and neurohormones have to be carefully monitored closely when using angiotensin receptor blockers, aldosterone antagonists, beta-blockers, natriuretic peptides, vasopressin antagonists, tumor necrosis factor inhibitors, and neutral endopeptidase inhibitors[14]. Surgical treatment of CVD involves the implantation of mechanical devices, surgical alterations of the heart, and in more extreme cases, heart transplantation. The current approach in the surgical treatment of CVD includes changing the shape of the left ventricle, the implantation of ventricular assist devices (VDA), and pacemaker and implantable cardioverter defibrillator (ICD) device implantations[14]. After a patient has undergone coronary artery bypass due to ischemic heart failure, they experience a reduction in

end-systolic volume. This makes it necessary for patients to undergo survival ventricular reconstruction in addition to the coronary bypass for the regulation of end-systolic volume[18]. Until recently, VADs could only be implanted into patients whose chest was large enough to fit the device and had end-stage heart failure[19]. Today, VADs can be implanted into either the right (RVAD) or left (LVAD) ventricle and the implantable unit can benefit patients with earlier stages of heart failure[19]. Pace makers and ICDs are regulatory devices that support the heart's electrical system, and regulate the heart's rhythm[20, 21]. The methods of treating CVD are not methods that restore heart function to infarcted areas, but rather support the damaged heart as a whole. Clearly there is a need for the development of a new therapy for CVD that is not as taxing on the heart as current treatment methods, with iPSC's being a likely candidate.

2. Materials and Methods

We obtained the information for this project and conducted our research primarily through literary review. Books, journals, articles, and actual copies of clinical trials were used for various aspects of the project. There were also interviews conducted with people who work in the field of stem cells. These people included professionals, students, doctors, and professors.

2.1. Interview Researchers

The following questions were asked when talking or interviewing individuals that are knowledgeable in the respective subject area.

1. Do you believe that cardiac stem cells are a viable option to repair damaged tissue in the heart?

2. Have you had any specific experience using a particular type of stem cell for heart repair?

3. If so, what types of cells have you found to be most effective in heart repair?

4. What stage is iPS cell research and development in at the current point in time?

5. Are there any potential drawbacks to creating and using iPS cells in the heart?

6. What are all of the costs (time, money, etc.) involved in creating iPS cells?

7. How far away is this type of research from human clinical trials?

8. How do iPS cells compare to cardiac stem cells in terms of effectiveness and cost?

2.2. **Market Analysis**

The main parts of the market analysis have to do with target market, operating market and the competitive strategy of a business. In the case of this project, the primary focus will be on the operating market.

Target market:

The target market consists of individuals that will purchase the stem cells. The considerations for each patient include age, gender, income level, social class, and education. All these factors affect the purchasing quantity of the stem cells for each patient. These target market characteristics were all taken into account when determining the level of revenue.

Operating market:

Operating market looks at the field of Biology as a whole, not focused around stem cells specifically. This includes individual companies that will be manufacturing the stem cells for application towards the target market. The attributes considered for the operating market include the industry standards and fluxes, the size and purchasing power of each firm, and the quality of the technology being produced relative to the price structure.

Competitive Strategy

The competitive strategy looks at analysis of a field of market from the business point of view. The tools used to analyze the competitive strategy of a stem cell producing firm was a SWOT analysis and the business strategy and its applications

2.3. **Current Trends in Market**

Looking at the Current trends in the Biology and health care industry our project will address the following questions.

1. Are there any barriers that may hinder entrance into the market?
2. What is the window of opportunity to enter the market?
3. Are there any indirect or secondary competitors who may impact your success?
4. What obstacles exist in the market that need to be addressed in order to achieve success (e.g., changing technology, high investment cost, lack of quality personnel, ethics)?

3. Discussion

3.1. Overview of Potential Stem Cell Types for Repair of Myocardial Infarction

3.1.1. Stem Cell Types

There are many types of stem cells that could have viable use for repair of a myocardial infarction. These include embryonic stem cells, mesenchymal stem cells, and cardiac stem cells. Embryonic stem cells are undifferentiated, pluripotent cells derived from the embryo of a fetus prior to differentiation. Currently most methods for harvesting embryonic stem cells are controversial due to various legal and ethical issues. Consequently, we do not believe that there is a very large market for these cells at this time.

Mesenchymal stem cells are most commonly classified as "bone marrow" stem cells, but similar types of stem cells are also found in adipose, umbilical cord blood, skeletal muscle, and other tissues[9]. Recently there has been some very promising research showing that these stem cells could be utilized for regenerative medical purposes, including, but not limited to treatment of tissue damaged during myocardial infarction. The International Society for Cellular Therapy (ISCT) has defined human mesenchymal stem cells, or hMSC's, by a series of criteria.

First these cells need to have a presence of certain cell markers. Cell markers are commonly named based on their clusters of differentiation (CD) followed by a number based on the order of when they were discovered. Clusters of differentiation are defined as clusters of antigen that are present on the surface of the cell. Cells that display a presence of cell markers CD73, CD90, and CD105 (in vitro) fulfill the first classification criteria. The second criteria say that they must display less than 2% of cell markers CD45, CD34, CD14, and CD19[9]. This is because these cell markers are associated with other tissues in the body that are not derived from bone marrow. Lastly a human mesenchymal stem cell must show an adherence to plastic and have multi-lineage potential meaning that they could develop into ectoderm, mesoderm, or endoderm[9].

At this time most testing of hMSC's has only been done in vitro, but one clinical trial investigating their effectiveness in the treatment of a myocardial infarction showed promising results. A group of 53 heart attack patients, whose hearts were injected intravenously with MSCs shortly after infarction experienced an improved heart and lung function[9]. More specifically three randomized concentrations of stem cells (0.5 million, 1.6 million, or 5 million MSCs/kg respectively) were administered into patients and compared against a placebo. Effectiveness was measured using echocardiography and the results showed a large improvement in ejection

fraction, or the amount of blood pumped from the heart in a single heartbeat, in hearts that received the stem cell treatments. Despite this there still needs to be more research conducted to determine the ideal concentration of mesenchymal stem cells to use. Furthermore there has been research that suggests that these stem cells tend to be very inefficient at targeting injury sites in the heart for various reasons such as becoming entrapped in the lungs before they reach the target site[10]. This has led to the theory that these hMSC's ability to heal injured tissue may not be due to differentiation into cardiac tissue, but instead may be caused by their secretion of factors that induce repair mechanisms such as angiogenesis[9]. Based on the reasons above we did not believe that hMSC's would be the optimal stem cell type for heart repair.

Until recently, the heart was considered to be post-mitotic and unable to regenerate. Researchers have identified human cardiac progenitor cells that could be used in the rebuilding of generating cardiomyocytes and coronary vessels[4]. These cells have mostly been found to differentiate into cardiomyocytes, but may also become smooth muscle cells and endothelial cells[5]. Recently the heart has been identified as an organ regulated by stem cells. Located in a "stem cell compartment" inside of the heart, human cardiac stem cells, or hCSC's have been discovered to be involved in postnatal development as well as the regulation of homeostasis in adult hearts[11]. Initially hCSC's were classified as bone marrow stem cells that had made their way into the heart, but after some closer analysis it was shown that this was not the case. These cells were shown to co-express the stem cell antigen c-kit as well as some myocyte transcription factors. Importantly, these cells did not contain CD45 or KDR which are common bone marrow cell markers[11]. This shows that hCSC's are not the same as bone marrow stem cells.

In trials on mice, hCSC's have shown some promising results. Infarcted mouse hearts were injected with hCSC's shortly after cardiac arrest which resulted in the generation of a

chimeric heart that included human myocytes and coronary vessels[11]. This regenerated tissue was shown to cause improvement of ventricular function and increased ejection fraction, despite the fact that these human tissues were formed independently of mouse tissue and that no cellular fusion had occurred. Although resident CSC's become present after a myocardial infarction, they typically have little to no regenerative effect because the majority of them in the damaged portion of the heart die along with the other cells that are damaged[11]. Other limitations of these stem cells are that they are limited to a compartment in the heart therefore their availability is relatively scarce. There are also a lot of unanswered questions about hCSC's such as their in vivo capabilities that need to be answered before more clinical trials should begin.

3.1.2. iPSC's

In 2012 Dr. Shinya Yamanaka and Dr. John B. Gurden were awarded the Nobel Prize in medicine for discovering how to turn adult epithelial cells into embryonic-like stem cells[7]. These cells are known as induced pluripotent stem cells. Induced pluripotency is achieved by using a virus to cause the overexpression of four cellular transcription factors: OCT3/4, SOX2, CMY-C, and KLF4. Evidence has been shown that there are also four alternative transcription factors that can lead to cellular reprogramming as well. These are Oct4, Nanog, Lin28, and Sox2; although further research is still needed to better understand these factors[12]. Usually after a few weeks of infection with the retrovirus, adult somatic cells begin to change their structure and express a human embryonic stem cell genetic profile[8]. For the purposes of this paper we have decided to use iPSC's to conduct our market analysis because we believe that they offer the most promise for the repair of damaged tissue in an ischemic heart after infarction. We also believe that these cells would be the best candidate for eventual mass production.

Induced pluripotent stem cells have been tested to treat sickle cell anemia, Parkinson's disease, and hemophilia in the past but have just recently begun to be studied in cardiac medicine[13]. In one study conducted on mice that compared iPSC treatments versus fibroblast treatments to repair an acute myocardial infarction, iPSC's proved to be very effective. The treatment involved the "intramyocardial transplant" of 200,000 iPS cells into each heart and resulted in overall significantly improved heart function compared to hearts that were treated with cardiac fibroblasts[13]. This restored function was classified as improved fractional shortening, lack of aneurysmal formation, and an absence of severe wall thinning[13]. Even though iPSC research is still in its preliminary stages, iPSC's have been shown to be effective in the treatment of myocardial infarction.

There has recently been an influx of new information in the scientific community regarding iPSC generation efficiency. One of the more interesting areas of research is the effect of microRNAs on the induction process. Micro RNA, or miRNA, are short, single stranded RNA that assist in various reactions in the body. It has been discovered that during the early stages of reprogramming three specific miRNA clusters are induced: miR17~92, miR106b~25, and miR106a~363. Simultaneous to the induction of these miRNA's, certain tumorigenic inhibitors of reprogramming: Tgfbr2 and p21 become present as well[12].

Table 1

Inhibitors and Aids of Reprogramming

Reprogramming Inhibitors	Reprograming Aids
Tgfbr2	miR-106b
p21	miR-93

The table above shows the reaction inhibitors and the micro RNA that silence them during reprogramming, thereby aiding in the efficiency of the induction process.

The presence of the inhibitor p21 is believed to be caused by a misexpression of Klf4 and cMyc. Inhibitor p21 promotes cell cycle arrest during iPSC induction, thus having a negative effect on the efficiency of the reaction. Tgfbr2 also acts as an antagonist during induction. Specifically miR-93 and miR-106b have been shown to enhance reprogramming efficiency by silencing these inhibitors[12]. By adding more of these miRNA during the reprogramming process scientists have been able to increase iPSC generation 4-6 fold[12]. As an aside, it is interesting to note that p21 has been discovered to be targeted by miRNA from the miR106b~25 cluster in cancer research as well[12]. Although there is still much more to learn about the induction of pluripotent stem cells, there is hope that learning to utilize miRNA's during the process could someday play an integral role in the mass generation of iPSC's.

3.1.3. iPSC potentials

One of the biggest downsides of organ and tissue transplants is that they are limited in their aspects of availability. Biocompatibility of the transplanted organs also becomes a serious concern since most donors are not related. Rejection of the organ by the recipient is a very serious threat, making life-long treatment with immunosuppressive drugs necessary. Induced pluripotent stem cells avoid these complications because the tissue or organ would be generated from the patients' own cells[22]. The potentials for iPSC's include the repair of disease-causing mutations and drug development[22]. Repair of genetic and degenerative disorders can be achieved through transplantable cells in which the gene expression is changed[23]. As previously mentioned, iPSC's have been shown to effectively treat genetic disorders, specifically sickle cell anemia and hemophilia, in mice through gene targeting[22,23]. Endothelial cells were cultivated from mice with sickle cell anemia and hemophilia, reprogrammed into iPSC's using gene targeting, and

transplanted into the mice[23]. The resulting reprogrammed healthy progenitor cells produced equally healthy red blood cells, and consequently cured the diseases[22,23]. This method can be replicated with other genetic disorders and could one day be applied to human trials.

Drug development through cultures of iPSC's is an area of research that shows great potential as well. This is due to the wide differentiation capabilities of induced pluripotent stem cells in vitro[22]. Many degenerative diseases are challenging to study and treat due to the limited accessibility of the affected tissues. Induced pluripotent stem cells have the potential to be differentiated into diseased cells, thereby eliminating the need for biopsied cells. Using iPSC's to study these disorders can also allow for diseases to be studied at earlier stages. This is because in most scenarios by the time a patient's tissue is studied, the disease is already well into its later stages. Scientists believe that iPSC's could be differentiated into diseased cells and will behave the same way as regular somatic cells going through the same stages of infection. It is evident that iPSC's could potentially be utilized in the identification of new drugs to treat degenerative diseases without surgical intrusion into the patient or constant biopsies[22]. Recent clinical research has already shown promise in treating patients suffering from spinal muscular atrophy (SMA), familial dysautonomia, and multiple lentigines syndrome using patient derived iPSC's to recapitulate the disease[22].

3.1.4. Current complications with iPSC's

In order for these induced pluripotent stem cells to be integrated into wider clinical and human research applications, there are issues that still need to be addressed:

- The creation of pluripotent cells from donor epithelial cells using manipulation techniques is less efficient in humans than in the mouse, blocking the integration of iPSCs into medical consumer markets for gene targeting[24].

- iPSC's still cannot be generated without alterations to the genome
- Not all cell types have been shown to be able to be differentiated from induced pluripotent stem cells
- Quality assessment of in vitro derived cells versus in vivo derived counterparts
- Efficiency and methods for generating iPSC's in a high-throughput and high quality manner[24].
- Residual oncogenetic factors could be expressed in the transplant patient, leading to cancerous tumors.

3.2. Marketing Analysis:

A market analysis is used to look at the potential feasibility of using therapeutic induced pluripotent stem cells to treat cardiovascular disease as regenerative medicine. The market analysis uses several tests to determine the current state of the biotechnology and medical markets and how the introduction of a new product will affect these markets.

3.2.1. Industry Analysis

The industry analysis looks at the current state of the market, looking at its present size, projected growth rates, trends and characteristics. In the case of induced pluripotent stem cells several industries have to be considered, such as the medical/healthcare, biotechnology and pharmaceutical[6]. These three industries all play a role as the medical industry will use stem cells to treat patients, the biotechnology industry will produce them, and the pharmaceutical will sell them.

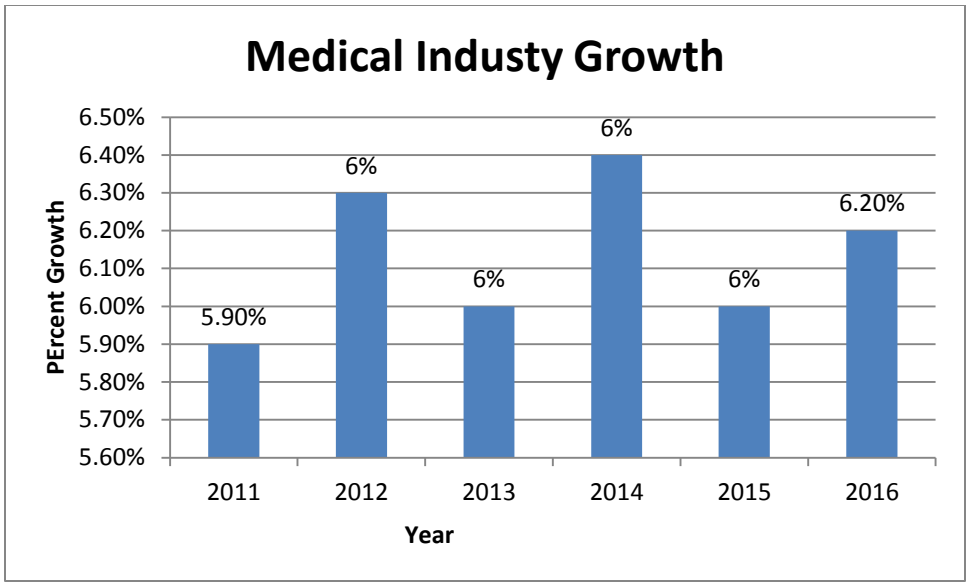

Figure 1 Medical Industry Projections

Figure one looks at the projected growth by year in the medical industry. The medical and healthcare industry represents consumer spending on healthcare and health services, such as medical practices, hospital fees and other human health fees. . This market is expected to grow 5-6% ever year for the next four years[6]. Some of the factors that are hindering the growth rate include insurance companies' attempts to control the medical costs.

3.2.2. Stakeholders Analysis

A stakeholder's analysis looks at the groups that have both power and influence over a given product in the market. The analysis directly looks at who has the most power to shape the products and who will influence its success. An X-Y Axis is used to demonstrate power and influence.

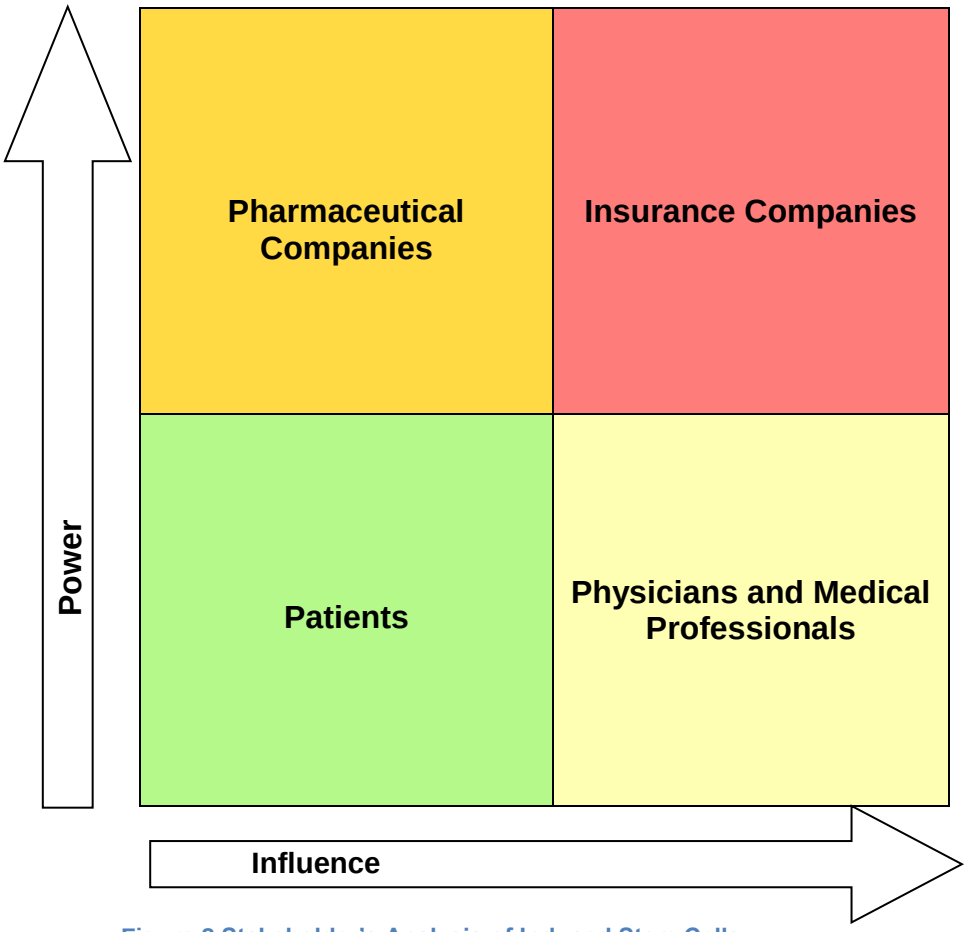

Figure 2 Stakeholder's Analysis of Induced Stem Cells

Figure 2 looks at the direct power and influence that insurance companies, pharmaceutical companies, medical professions and patients have over the commercialized sale of iPSCs on the market. Insurance companies have a high level of influence and power, as they will cover the costs for the patients receiving treatment. Pharmaceutical companies are placed in the high power group because they will most likely sell the product to the patients. Physicians and medical professionals tend to have a high level of influence because they will be using the product, but they are limited to using medical procedures that are covered by insurance since this is how the majority of consumers pay for healthcare. Patients are low power and influence, they are people that need to be considered in the making of the product, but in the end have no power to change the product sales, or influence in changing the treatment methods.

3.2.3. Target Market

The target market analysis looks at what group of consumers the iPSC's are marketed towards. There are several key factors to consider when looking at stem cells. First and foremost is the use of the product. There are several key practices that scientists and healthcare professionals will use stem cells for including medical research, genetic disease treatments, regenerative tissues, and drug research. The resulting effects of this new technology have the potential to be revolutionary as numerous diseases formerly termed “incurable” would be treatable with the development of fully induced pluripotent stem cells[25].

There are several key customer groups that will affect the development of marketing stem cells. The first major customer has to be insurance companies, because chances are that the end product will be too expensive for the general public to purchase, at least in the starting stages of production[29]. Scientists, drug development companies, and wealthy consumers who could bypass insurance companies to purchase iPSCs on their own are also part of the target market.

3.2.4. Cost Analysis

Therapeutic iPSC have large variable costs associated with taking cells from the patient, in the form of a biopsy and reprograming those cells into pluripotent stem cells[27]. Determining the cost of all the supplies was based on the following production line and the recommended supplies for these processes. The list price was used and discounts based off of mass production were not considered, as therapeutic iPSCs for cardiac treatment are not currently ready for mass production[27].

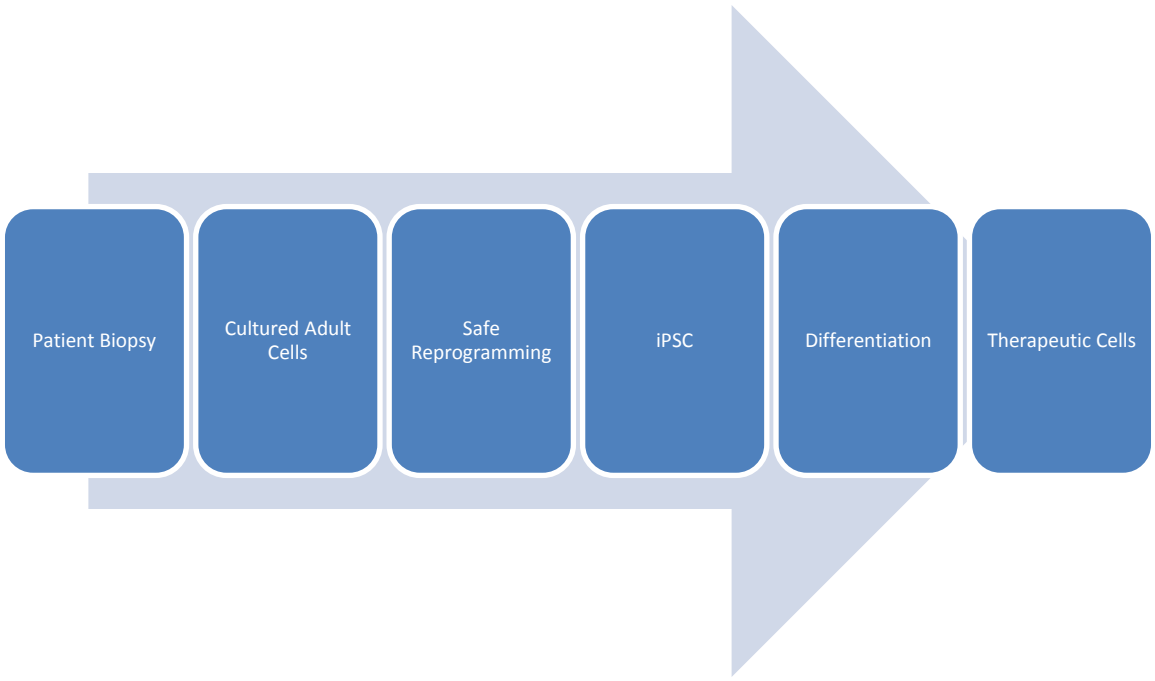

Figure 3 Process of Therapeutic iPSC

The income statements of several mid-sized biotechnology companies were used to examine the fixed cost with the exception of specific equipment which is unique to the iPSC process. Research and development are essential to a biotech company which is why almost 50% of total costs were used for this business discipline. The fixed costs in this analysis are also based off of the first month, as some of the items are one time costs and others may vary month to month (research and development). The cost for one employee was determined based on the hourly rate for an employee with four to eight year experience in the field of biotechnology[28]. General administration costs take into account the wages of non-hourly employees of the company who are not working directly on the project, but are still doing related activities.

Cost Induced Pluripotent Stem Cells	Cost
Variable Costs	

Patient Biopsy	$ 360.00
Biotech Employee @ $23.22 per Hour	$ 115.00
Materials	
CytoTune®-iPS Sendai Reprogramming Kit	$ 2,055.00
Episomal iPSC Reprogramming Vectors	$ 755.00
TaqMan® iPSC Sendai Detection Kit	$ 350.00
Serum Replacement	$ 30.00
hESC SFM	$ 260.00
Essential 8 Medium	$ 195.00
XenoFree ESC/iPSC kit	$ 782.00
Neurobasal™-A Medium	$ 58.00
N-2 Supplement	$ 69.00
G-5 Supplement	$ 57.00
ELF® 97 Endogenous Phosphatase Detection Kit	$ 350.00
TaqMan® Array Human Stem Cell Pluripotency Panel	$ 1,539.00
Fixed Costs	
Research and Development	$ 22,000.00
General Administration	$ 5,240.00
Rent	$ 1,500.00
Utilities	$ 650.00
Equipment	
Neon® Transfection System	$ 6,975.00
Other	$ 4,500.00
Total Costs	$ 47,835.00

Figure 4 Total Cost of Producing Induced Pluripotent Stem cells of Therapeutic Quality

Figure 4 determines that the total cost for producing one round of therapeutic quality stem cells would be $47,830.00[28]. This cost, although fairly accurate, can not be a perfect estimation as this technology is not currently on the market for mass production and some of these numbers are estimates.

After the total cost was calculated, the cost for units produced was displayed in increments of 50. A large number was used due to the large number of cardiac problems per month in the United States. On Average, there are 77,900[1] heart problems per month. Induced

pluripotent stem cells have the potential to be the most effective treatment for damaged cardiac tissue. For this analysis, it will be assumed that one employee could handle 25 samples during one cycle.

3.2.5. Break Even Analysis

Income Statement		Amount $
14 Units Sold		
Revenue	$	140,000.00
Variable Costs	$	97,565.00
Gross Margin	$	42,440.00
Fixed Costs	$	40,864.00
Net Income	$	1,575.00

Figure 5 Income Statement for Break Even Analysis Selling price at $10,000 per unit

A break-even analysis was used to determine the number of units that would need to be sold to turn a profit. A unit is considered one group of therapeutic stem cells that could be used to treat patient's cardiac problems. The price of one unit sold was set at $10,000 (see section 4.1.6). The main reason why the break even point is low, fourteen units, is because the gross margin for each unit sold is about $3,000 and the fixed cost is relatively low compared to the variable costs.

3.2.6. Pricing Strategy

Income Statement		Amount $
25,000 Units Sold	**77,900 Heart Attacks per month**	
Revenue	$	250,000,000.00
Variable Costs	$	174,219,500.00
Gross Margin	$	75,780,500.00
Fixed Costs	$	40,864.00
Net Income	$	75,739,636.00

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Figure 6 Income Statement for 50,000 units sold per month; selling price at $10,000 per unit.

A selling price of $10,000 was used during the break-even and mass production prices. The reasoning for this price to be used is because it would be comparable to a bypass surgery except that the 10-day hospital admission would be reduced to 3 days. Each hospital admission is $1800 plus $3,715 for physician's fee and $2,500 anesthesia services which adds to $11,610[25,28].

3.2.7. SWOT Analysis

A SWOT analysis (which stands for strengths, weaknesses, opportunities and threats) was used to look at a potential iPSC company's strengths and weaknesses in comparison to its main competitors. For the purpose of this analysis, we assumed that the company is the only company

that is selling iPSCs on the market and related it to other treatment methods from various other companies.

Figure 7 SWOT Analysis for iPSC product

The SWOT analysis in figure 7 indicated several benefits of iPSCs for producers of the treatment. The strengths of this new technology come from the fact that it has the potential for higher success in modern medical treatments as well as improved biocompatibility and reduced hospital fees compared to the current treatment options available. Current issues that could threaten the success of induced pluripotent stem cells are reluctant insurance companies, delays in research and funding, and the potential oncogenic nature of stem cells.

3.2.8. Treatment Cost Analysis

Treatments	In-hospital Costs	Pharmaceutical Costs	Total costs
iPSC	$ 10,000.00	$ 1,700.00	$ 11,700.00
1. Cardiovascular Disease			
a. Lifestyle Changes	$ -	$ -	
b. Medications	$ -	$ 1,700.00	
c. Surgery			
i. Coronary angioplasty	$ 22,768.00	$ -	$ 24,468.00
ii. Coronary artery bypass	$ 63,648.00	$ -	$ 65,348.00
2. Heart Defect Treatments			
a. Medications		$ 1,700.00	
b. Open Heart Surgery	$ 324,000.00	$ -	$ 325,700.00
c. Heart Transplant	$ 977,700.00	$ -	$ 977,700.00
3. Cardiomyopathy Treatments			
a. Medications	$ -	$ 1,700.00	
b. Medical devices	$ 22,735.00	$ -	$ 24,435.00
c. Heart Transplant	$ 977,700.00	$ -	$ 979,400.00
4. Valvular Heart Disease Treatments			
a. Medications	$ -	$ 1,700.00	
b. Valve repair	$ 35,976.00	$ -	$ 37,676.00

Figure 8 Treatment Cost Comparison

Figure 8 looks at all current forms of treatment on the market in comparison to iPSCs. Heart transplants and open-heart surgery are among the most expensive and invasive surgeries on the market. Stem cells will reduce the number of invasive surgeries performed each year which will shorten patient recovery times and improve patient turnover rates in hospitals[25]. Virtually all surgeries and treatments have pharmaceutical costs of some sort[26]. Pharmaceutical costs will remain with the new technology as well because iPSC treatment will only regenerate damaged tissue, not prevent the problems[29]. Although a combination of lifestyle changes and iPSCs would be the lowest costing treatment for a patient recovering from a myocardial infarction; short term medications still need to be taken after the initial heart attack to ensure that the healing process goes as planned.

Conclusion

With the right economic opportunities as described in this paper, Induced Pluripotent Stem Cells have a viable future in regards to advancing regenerative medicine for the application of cardiac repair. Because iPSCs are autologous and are not surrounded with political and religious polarization, they are optimal in treating infarcted areas of the heart. Due to the cells being autologous, availability for treating damaged areas in the heart will no longer be an issue, along with worries of biocompatibility and tissue rejection. Although research of iPSCs and their application in treating cardiovascular disease is still in the developing stages, it is showing promise for the treatment of myocardial infarctions. The current complications with developing iPSC are genetic in nature and have more to do with efficiency, the largest of which is the undesired expression of oncogenetic factors in the cell.

Current market research suggests that Induced Pluripotent Stem Cells have a high production cost, a reality that could inhibit the proliferation of this technology into current medical practices. If these cells could be mass produced and were FDA cleared for application towards treating cardiovascular disease, there exists a possibility of considerable revenue for a company that penetrates the market.

The promises held by iPSCs have not been fully ascertained, as everyday research is being done on their effects. Procedural practices, including medical, manufacturing, biological, and business procedures have not been perfected. The advances that these stem cells hold are innumerable, as they can be applied in the treatment of numerous other diseases. Over 700,000 heart attacks happen in the United States every year, a number that cannot be lessened with current medical and pharmaceutical technology. The hope of being able to treat all these infarcts lies with the development and propagation of Induced Pluripotent Stem Cells in current medical practices.

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