STEM CELL ENGINEERED PANCREATIC BETA CELLS TO COMBAT DIABETES

Kevin Pietz, kwp6@pitt.edu, 1020, Ben Wong, bmw98@pitt.edu, 1020

Abstract*—Type 1 diabetes (T1D) is a chronic illness that negatively impacts the daily lives of millions. This destructive autoimmune disease causes the elimination of pancreatic beta cells, necessary for insulin production, and the rise of high blood glucose levels. These are dangerous outcomes that can become fatal for diabetic individuals. Unfortunately, a cure to eradicate T1D remains elusive. A growing area of research that holds potential for a permanent solution to T1D is stem cell engineering. Stem cells have the ability to differentiate into any somatic cell within the human body. Utilizing this technology to develop engineered pancreatic beta cell replacements could render current treatments, such as multiple daily injections and insulin pump therapy, obsolete. Different types of stem cells can be cultivated to engineer these beta cells. Transcription factors are implemented in the stem cells in order to differentiate them into beta cells through viral vector delivery altering the cell's DNA. After beta cells are obtained, they are transplanted via the portal vein. On record, no clinical trials for this entire process has occurred. However, each individual step from stem cell cultivation to transcription factor integration to portal vein transplantation has been successful within their respective studies. This new stem cell engineering technology still has possible drawbacks such as economic outcomes and transplant rejection. In terms of ethics, the use of stem cells is faced with moral issues regarding human embryonic research and experimentation. The sustainability of stem cell engineering is shown by potential and future application in progressing medical treatments for diabetes and other chronic illnesses.*

Key Words—beta cells, cell differentiation, portal vein, retroviral vector, transcription factors, T1D

APPROACHING THE BATTLEFRONT OF DIABETES

Stem cell engineering has the potential to change a lifetime of blood-drawings and needles-injections into a onetime visit to the hospital for type 1 diabetics. For a disease that affects children significantly more often than adults, it is unfortunate that for decades now the main form of treatment involves daily syringe injections [1]. Current attempts at diabetes management involve daily injections or medical devices like the insulin pump, while alternative methods, including transplanting organs, has of yet to achieve widespread success. Advances in noninvasive treatment are constantly being searched for, and stem cell engineering provides a promising alternative to the daily finger-pricks and injections. By engineering stem cells with multiple transcription factors to secrete insulin, a solution with none of the drawbacks of current treatments lies within reach.

T1D: Causes and Effects

Type 1 diabetes (T1D) occurs following the autoimmune destruction of pancreatic beta cells, and is diagnosed by hyperglycemia, or high blood glucose levels [2]. Beta cells lie within the pancreas, a small organ that is a part of the endocrine system, which deals with hormone regulation [3]. Figure 1 below shows the cross-section of cells within the pancreas. The primary function of beta cells is to secrete the hormone insulin in response to high blood glucose levels. As insulin enters the bloodstream, it allows glucose to enter cells, bringing blood glucose levels down into a normal range [4]. This negative feedback loop requires no effort by healthy individuals, but for diabetic patients, maintaining healthy blood glucose levels is a challenging daily effort by the patient and their doctor.

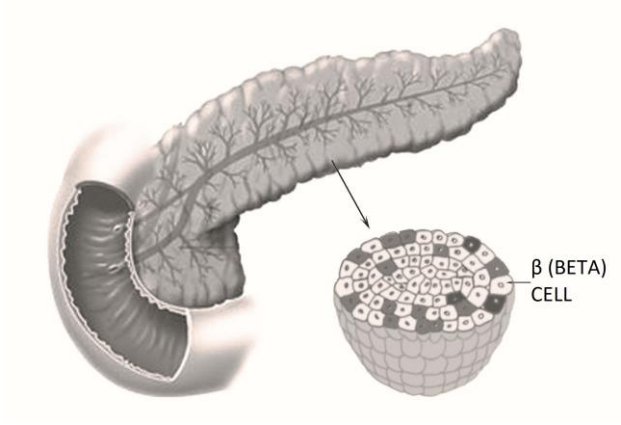

Figure 1 [5]
Pancreas and cell cross-sections.

If blood sugar levels are not strictly monitored, negative side effects accumulate over a patient's lifetime, often shortening it. Long term hyperglycemia leads to many complications throughout multiple parts of the body. Diabetic individuals with uncontrolled high blood glucose levels are at increased risk of heart attack and stroke, and the excess glucose takes an especially high toll on small and delicate blood vessels in the kidneys and eyes, leading to kidney damage and blindness [3]. The glucose damages blood vessels in the extremities, leading to loss of limbs in many patients who have lived with the disease for a number of years [3]. Apart from the long-term damage to major body parts, diabetics are at risk for short term dangers, also.

Without insulin to allow glucose to leave the bloodstream, cells lack their necessary energy supply. Cells must rely on alternative energy forms, such as breaking down fat storage. This process leads to a chemical byproduct of ketones, which are acidic chemicals released into the blood [3]. High ketone levels lead to a dangerous condition called diabetic ketoacidosis (DKA), causing individuals to fall unconscious, and if immediate action is not taken, they may die [3]. While deaths by DKA are rare, entering this condition leads to hospitalization for multiple days, extensively interrupting the lives of diabetics.

Considering how serious the implications of unmanaged diabetes can be, it is no surprise that many methods have arisen to combat the disease. However, even with numerous lifesaving therapies, there is no cure to T1D.

Traditional Treatments

A number of treatments exist for type 1 diabetic individuals, all with the common goal of regulating blood glucose levels with insulin. The most common forms of treatment for type 1 diabetic patients are multiple daily injections and insulin pump therapy [6], while beta cells and whole pancreas transplants are rarer [7].

Multiple daily injections and insulin pump therapy both require invasive blood glucose tests several times a day and involve pricking fingers with a small lancet. A strict diet is often required, and daily life is planned around managing the disease. Patients on multiple daily injections must carry syringes or an injection pen with them and inject insulin via subcutaneous needle during mealtimes and at times of high blood glucose levels [6].

Insulin pump therapy brings more control into diabetic patients' lives. These individuals carry an insulin pump, a small medical device about the size of a cell phone. The pump delivers insulin through a thin tube to an injection site leading under the skin, which must be changed every couple of days [6]. Advancements have been made recently in insulin pump therapy by the addition of the continuous glucose monitor. This device tracks glucose levels constantly, visualizing trends in real time, and in the Minimed 670g, will deliver insulin automatically in response to a detection of high glucose levels [8]. Even with the new advancements in insulin pump therapy, individuals must still monitor blood glucose for accuracy and deliver insulin at meals [8]. Although it is a big step in diabetic treatment, the new insulin pump system is far from a permanent solution.

Less common and more extreme forms of treatment are beta cells and whole pancreas transplants. These often occur in patients with poor management of blood glucose alongside kidney transplants [7]. While individuals no longer need to monitor their blood glucose levels, they must take immunosuppressive medicine for the transplant [9]. Rejection of transplant and a lack of available pancreas donors means this treatment occurs very infrequently. Other than these drawbacks, pancreas transplants are the closest treatment to a cure for diabetes, because it replaces the destroyed insulin producing beta cells with new ones.

Multiple daily injections, insulin pump therapy, and pancreas transplants all have drawbacks. Multiple daily injections and insulin pump therapy do not prevent negative side effects from long term hyperglycemia and cause major inconveniences in daily life [6], and there is a limited supply of pancreas donors for transplants [7]. These current treatments are not sustainable, so a new method of treatment is necessary, one that cures T1D and is widely available.

Treatments are deemed sustainable if they can combat diseases and improve health in the present, but also perpetuate development to advance overall quality of the medical field and health of future generations. Beyond being technologically feasible, medical treatments must be able to last both economically and socially. An economically sustainable medical technology is affordable to those who need it most. An issue that many innovative treatments face is social backlash from the public. If the public denounces stem cell therapy, its use will be limited, and the treatment will fail to last. Another aspect of sustainability comes from the United Nations' sustainable development goals for 2030. One goal is good health and well-being, including improving quality of life [10]. Even if a treatment extends the life-time of an individual, it could decrease quality of life. Current treatments of T1D follow this trend. While type 1 diabetics'

life expectancies have greatly increased, simply remaining healthy is a monumental task for patients utilizing existing therapies. As a result, a sustainable treatment is imperative to cure diabetes and improve quality of life.

Engineering stem cells with transcription factors has the potential to cure T1D. It is possible to use a patient's own stem cells and differentiate them into beta cells that do not trigger an autoimmune response, and secrete insulin in response to glucose without input from the patient.

THE MANY FACES OF STEM CELLS

Stem cell research has become a much more prominent branch of science over the recent years. Stem cells are known for their remarkable ability to develop into a multitude of different cell types in the body [11]. The potential of developing pancreatic beta cells for long-term replacement in diabetic patients can possibly provide a true cure for the chronic illness. The capability of renewing themselves for long periods of time and the ability to differentiate into specialized cell types are the main properties that give stem cells this potential [11]. There are different types of stem cells that can be cultivated. Even though they can be quite different, in the end, they all possess some sort of differentiability. In differentiating stem cells specifically into insulin secreting pancreatic beta cells, the advantages and disadvantages of each must be weighed, and one may stand out as the ideal stem cell for treating diabetic patients.

Human embryonic stems cells (hESCs) are the first to come into mind when stem cell research is discussed. These stem cells are predominantly used because of their known abilities and direct cultivation from embryos. The hESCs possess such abilities to differentiate into any somatic cell type because the stem cells are initially undifferentiated to eventually create a whole new organism [11]. These cells' ability to proliferate into many more stem cells while still remaining undifferentiated is an even more unique property [11]. This is beneficial, for it provides an abundance of undifferentiated cells that can later be tested or implemented to develop necessary somatic cells. Harvesting these undifferentiated cells is key in the technological process of developing pancreatic beta cells. The hESCs are cultivated by transferring embryos fertilized *in vitro* into culture dishes [11]. Embryos are maintained in controlled conditions for ideal growth and prepared for stem cell extraction from their germ layers [11].

Adult stem cells are like hESCs in the sense that they too are undifferentiated cells. However, the difference lies in their origin and differentiability. Adult stem cells are undifferentiated cells found among differentiated cells throughout a specific tissue or organ in the adult body [11]. These cells are primarily utilized for maintaining and repairing tissue in which they are found [11]. Additionally, adult stem cells tend to not possess the pluripotent nature (ability to become all various types of cells in the body [11]) of hESCs when it comes to differentiability: "Adult stem cells are thought to be limited to differentiating into different cell types of their tissue of origin" [11]. Even though there appears to be limitations on adult stem cells, they are still a type of stem cell that can fulfill the purpose of developing pancreatic beta cells.

There is one last type of stem cell that has only been around for about a decade: the induced pluripotent stem cell (iPSC). These stem cells are somatic cells that have been genetically reprogrammed back to a hESC-like state [11]. These offer another alternative for culturing stem cells. However, even though they meet the defining criteria for pluripotent stem cells, it is unknown if they differ significantly from hESCs in clinical trials [11].

The dedifferentiation of somatic cells and the differentiation of stem cells are not merely superficial processes but involve altering biological code. It is all carried out through the implementation of special gene expressive proteins called transcription factors.

UTILIZING TRANSCRIPTION FACTORS FOR DEDIFFERENTIATION AND DIFFERENTIATION

What Are Transcription Factors?

While stem cells have the potential to differentiate into any type of cell, that process must be directed in some manner. The process of engineering stem cells into pancreatic beta cells mimics the development of these beta cells in embryos. Thus, studying embryonic development helps direct scientists and engineers on how to replace destroyed cells.
In developing embryos, the genetic code and regulation of gene expression determine what role each cell will play in the mature human body. Gene regulation occurs when certain genes are expressed more or less than others, so individual cells can perform different functions [4]. In a developing organism, genes are regulated to differentiate the cell, a process driven by transcription factors. Transcription factors are proteins that influence this specialization of stem cells by binding to genes in a cell's DNA sequence [4]. Once bound, these regulatory proteins will influence whether a certain gene or set of genes is expressed or not through positive or negative regulation respectively. These genes are translated into ribonucleic acid (RNA, a similar molecule to DNA) strands, which are translated into proteins by ribosomes [4]. These newly expressed proteins perform functions around the cell, and in stem cells, they will differentiate the cell. In the case of iPSCs, transcription factors can be used to dedifferentiate somatic cells into iPSCs to later be differentiated by the same process as differentiating hESC and adult stem cells [12].

Figure 2 depicts the overall process inside a cell started by transcription factors at which genes are turned into proteins to perform specific cell functions. The first step involves transcription factors binding to DNA causing desired genes to

be transcribed into RNA. This RNA is then translated into proteins for cellular functions.

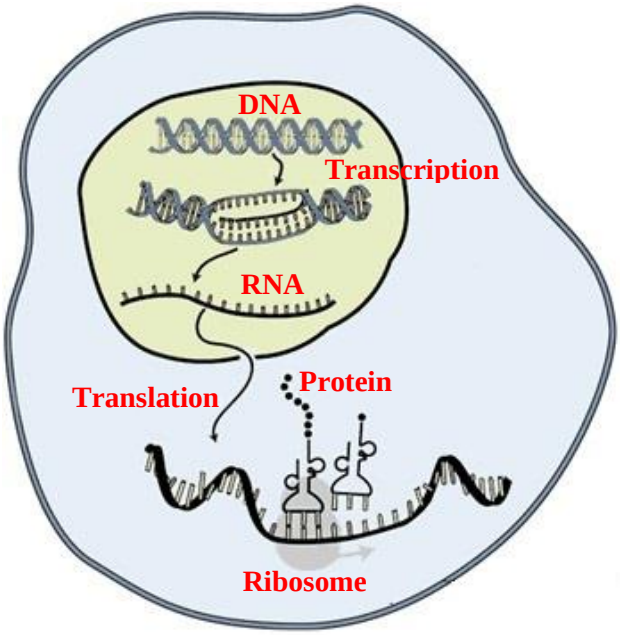

Figure 2 [13]
Transcription factors altering genetic code.

Dedifferentiation of Somatic Cells to Develop iPSCs

Before the differentiation process is discussed, it is necessary to explain the dedifferentiation process to develop iPSCs from somatic cells. This process involves the combinations of Oct3/4, Sox2, c-Myc, and Klf4 transcription factors [12]. Oct3/4 and Sox2 have been experimentally shown to maintain pluripotency and function as core transcription factors [12]. This brings somatic cells back to a state that further resembles that of hESCs. Even more efficient, the c-Myc factor specifically enhances proliferation and transformation [12]. This mimics the abilities of hESCs. The last transcription factor Klf4 represses other proteins, such as p53, that would inhibit the necessary factors to dedifferentiate [12]. However, Klf4 may act as an antiproliferative [12]. This property can be canceled out by c-Myc's proliferative property. As a result, both transcription factors must be present for dedifferentiation. All four of these transcription factors are necessary for dedifferentiation of somatic cells to be later differentiated into pancreatic beta cells.

The Transcriptional Route to Insulin Producing Cells

Differentiating a stem cell, regardless of whether it is a hESC, adult stem cell, or iPSC, is more than a simple step of using one transcription factor. It involves a multi-step process of various transcription factors, with one factor expressed after another. The pancreatic and duodenal homeobox gene 1 (Pdx-1) transcription factor is used as the initial step towards beta cell differentiation: "[Pdx-1] activation is the first sign of specification toward pancreatic fate" [14]. Utilizing this transcription factor starts a path that is already catered to pancreatic cells, and as a result, insulin producing beta cells. Pdx-1 leads to the next transcriptional step of determining endocrine or exocrine specification [15]. These two types of specification are related to whether a gland secretes directly into the bloodstream or not [14].

Neurogenin 3 (Ngn3) is one of the important transcription factors to begin cell differentiation. Ngn3, part of a transcription factor family that regulates the determined fate of cells, is specifically catered to endocrine cells within the pancreas [14]. Beta cells secrete insulin directly into the bloodstream, so endocrine specification is the route to take. This directly correlates with the technology of engineering pancreatic beta cells that could replace those destroyed due to T1D. Creating a baseline with Ngn3 allows following transcription factors in the process to differentiate the cells into more accurate beta cells.

The Ngn3 factor not only differentiates cells into endocrine cells, but also promotes the expression of the Pax4 gene. Once expressed, the Pax4 factor is vital for the specialization of beta cells [14]. Pax4 has been experimentally determined to drive differentiation towards a beta cell phenotype [15]. Specifically, in an experiment involving rodents, "Pax4 deficiencies in mice results in the lack of beta and gamma cells; therefore, it can be deduced that Pax4 is required for determining beta cell fate" [15]. This is key in engineering beta cells to treat diabetes. Using even more specific transcription factors throughout the multi-step process can likely develop the most efficient beta cell replacements.

The process of beta cell differentiation continues to get more specific because Pax4 promotes additional transcription factors. This promotion is due to the fact that Pax4 mediates the differentiation of both gamma and beta cells in the pancreas, and additional transcription factors are implemented to favor beta cell differentiation [15]. The final step to differentiate to a beta cell phenotype is governed by Nkx2.2 and Nkx6.1 transcription factors [15]. The removed use of Nkx6.1 results in the absence of beta cells, but all other pancreatic cell types develop [15]. However, when Nkx2.2, which is expressed in other pancreatic cells types such as alpha cells, is removed, insulin-producing cells become absent [15]. As a result, both are needed to differentiate beta cells properly. According to specialists from the University of California San Francisco, "Nkx6.1 affects cells in transition from early Ngn3-expressing precursors to differentiated beta cells. Because Nkx2.2/Nkx6.1 double mutants display the same phenotype as Nkx2.2 single mutants" [16]. Therefore, Nkx6.1 is considered downstream and must follow after Nkx2.2. These last transcription factors are most vital to complete the differentiation process to generate the necessary beta cells.

Figure 3 summarizes the overall differential route to beta cells via the respective transcription factors shown in yellow boxes. Each transcription factor discussed corresponds with their respective step in the differentiation process briefly described to the left.

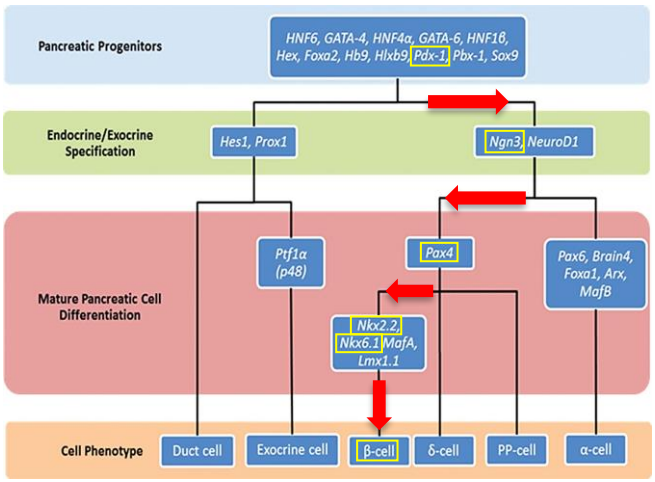

Figure 3 [15]
Multi-step process of transcription factors catered to beta cell phenotype.

Viral Vector Delivery

In order for transcription factors to perform their roles within a cell, they must first enter that cell and incorporate into the DNA sequence to be expressed. One common method used in genetic engineering today is viral vector delivery. Vectors are anything that transports genes into a cell, and viruses are specialized for injecting viral genetic code into the DNA of a host cell [4]. Scientists have safely harnessed this power by replacing viral genes with desired genes, such as transcription factor genes. The viruses can then incorporate this DNA into a stem cell without the negative side-effects from regular viruses [15]. While it may sound dangerous to use viruses, a common cause of great sickness, they have safely been used in genetic engineering for years.

Viral vector delivery is so commonly used, that there are numerous types of vectors derived from different viruses. Some examples include adenoviral, lentiviral, and retroviral, each one better in one case or another [15]. In the case of delivering transcription factors to stem cells, retroviral is the desired viral vector for its numerous advantages. This vector is capable of carrying more genetic code than other vectors and needs to inject four different gene sequences for the four transcription factors [15]. The retroviral vector has no expression of viral proteins, meaning it will not trigger an immune response, which is important when transplanting into the human body [15]. By using viral vectors to inject transcription factor genes into stem cells, the genes can be expressed. The transcription factors can differentiate the stem cells into pancreatic beta cells outside of the human body.

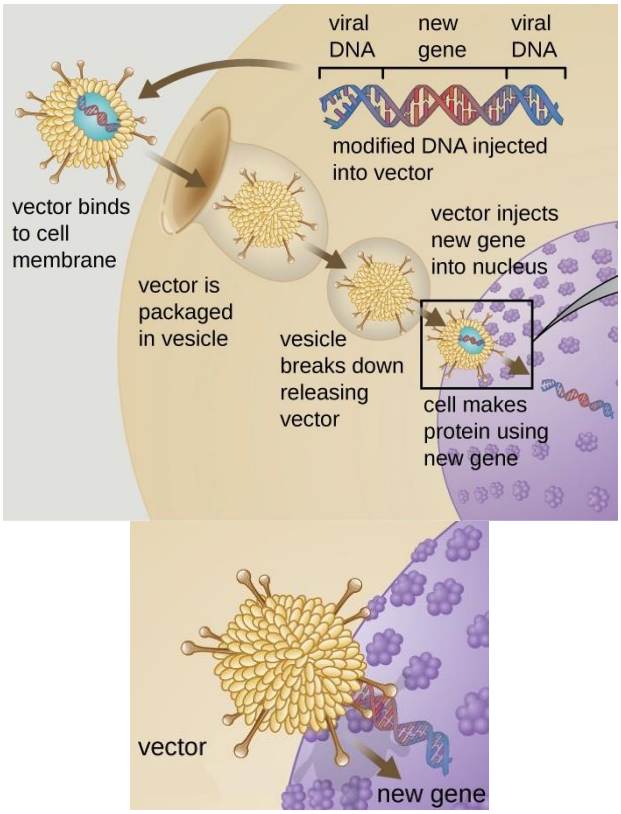

Figure 4 [17]
Viral vector injecting a foreign gene.

THE PORTAL TO TRANSPLANTATION

After the stem cells are treated with transcription factors and allowed to differentiate into insulin producing beta cells, the last step is to transplant those cells back into the patient's body. This is performed through surgery, where the recently differentiated beta cells are infused into the body via the portal vein, a vein connecting the pancreas to the liver [9]. In non-diabetic individuals, insulin is secreted by the beta cells of the pancreas and enters the bloodstream by means of the portal vein [7]. By injecting the beta cells via portal vein, the cells enter the liver, from which they will secrete insulin [18]. This is a common form of surgery for transplant of donor islet cells. Once the transplant is complete, and the islet cells are now injected into the patient's body, the differentiated cells act as replacements for previously destroyed islet cells by secreting insulin in response to glucose.

THE APPLICATION OF ENGINEERED STEM CELLS

On record, no clinical trials for engineering stem cells into insulin secreting beta cells have occurred. However, the individual steps to this process have all passed clinical trials. As a result, one can infer the overall process is likely to be

successful. Many experiments and clinical trials for stem cells, the use of transcription factors, and beta cell transplants have yielded positive results within their respective paradigms.

Clinical trials for stem cells have occurred successfully for years, treating many different types of diseases in patients [19]. Stem cells have been used in clinical trials to repair damaged heart muscle, multiple sclerosis, and sickle cell disease [19]. These diseases share something in common with T1D in that they involve damaged or destroyed cells. These clinical trials show a promising future for the use of stem cells in treating T1D.

The use of transcription factors to produce insulin secreting cells has also shown promising results. According to Dario Gerace, a researcher at the University of Technology Sydney, his lab used transcription factors to modify liver cells into insulin producing cells, reversing hyperglycemia [15]. This research indicates transcription factors are a viable tool for solving T1D. Moreover, integrating the use of stem cells, which are more prone to change than liver cells, to engineer beta cells can be more effective.

The final step transplants the differentiated cells via portal vein, which has occurred in beta cell transplant surgeries in recent years. Multiple trials of this T1D treatment have successfully injected insulin producing cells into the body, allowing patients to become insulin independent [9]. Considering both treatments transplant cells via portal vein, the success of beta cell transplants indicates success in injecting insulin producing cells for engineered stem cell treatment.

Each step to engineering stem cells into beta cell replacements has undergone successful clinical trials. Even though no clinical trials of specifically differentiating stem cells with transcription factors into insulin secreting cells have occurred, proof that each step individually is successful suggests the overall process will yield positive results.

THE OBSTACLES OF APPLICATION

Every medicine and cure has its drawbacks, and it is important to consider possible obstacles when developing new medicines and therapies. Some issues may arise that could prevent the success of stem cells as beta cell replacements, and some issues prevent its use now.

A common roadblock to a great idea is often the expenses. Even if stem cell engineering has a high success rate and effectively cures diabetes, if this method is too costly it will prevent widespread availability. An insulin pump is an expensive form of treatment, and without proper health insurance, many individuals favor multiple daily injections for reduced prices [20]. A similar problem may arise with stem cell engineering.

The final step in engineering pancreatic beta cells, the transplantation of differentiated cells via portal vein, comes with costs that may inhibit many from undergoing the procedure. This step is similar to the traditional method of beta cell transplantation, which many Medicare and Medicaid patients are currently unable to undergo due to reimbursement issues [21]. This could mean that many individuals would not have access to the innovative treatment of engineering stem cells to treat T1D. For a technology to be economically sustainable, it must be affordable to those who need it.

Transplanting through the portal vein still holds an additional complication. A high rejection rate for the transplant would mean an ineffective treatment, even after successful earlier steps. In traditional islet cell and pancreas transplants occurring today, numerous immunosuppressants must be taken to prevent this rejection, causing more inconveniences to diabetic individuals [9]. However, the stem cell engineering approach overcomes this hurdle from the very beginning of the process. Organ rejection occurs because the transplant is seen as a foreign entity by the body's immune system. Antigens on the surface of cells act as name tags for white blood cells to recognize foreign or familiar entities, and if an organ came from another body, its antigens will be recognized as foreign [4]. If the stem cells used to differentiate into beta cells were originally hESCs, they could potentially set off an immune response after transplant. IPSCs are different from other stem cells in the fact that they originally come from somatic cells of the patient them self [11]. This means they will have antigens that do not trigger an immune response, leading to a successful transplant with no need of immunosuppressants.

Another possible problem that could arise during the implementation of engineering stem cells comes from the cause of T1D itself. As previously stated, T1D is an autoimmune disease where the body attacks its own cells, specifically the beta cells [2]. By adding in another set of beta cells, wouldn't the immune system simply attack the beta cells again? While it has not been shown in experiments up to date, engineering stem cells has the ability to remove the risk of an autoimmune attack. The very idea of stem cell engineering allows for the cell to be differentiated to the specifications of the scientist or engineer. When the specific cause of T1D is better understood, steps can be taken to engineer a stem cell that produces insulin but does not trigger an autoimmune attack like the destroyed beta cells.

The main reason stem cell engineering is not a treatment available for type 1 diabetics is because it is such a new innovation that it is still in the earlier stages of development. New treatments and medicines must go through rigorous experiments and trials, and stem cell engineering for beta cell replacements has not had any clinical trials as of now. There have been lab experiments of isolated stem cells secreting insulin in response to glucose, and there have been numerous successful beta cell transplants. So, there exists proof of concept for this innovation, but it remains in its developmental stages.

THE ETHICAL DILEMMA OF USING STEM CELLS

Ever since the beginning of stem cell research, controversy has erupted relating to the ethics of testing stem cells. Specifically, hESCs are the topic of debate. Heated opposition has emerged because the harvesting of hESCs involves eliminating the human embryo after stem cells are extracted [22]. Adding to the ethical dilemma, the debate of the beginning of human life and the value of embryonic life comes up as well [22]. Even though opponents argue that hESC research is immoral, they neglect the advancements in utilizing other stem cells. That is not to say, however, that hESCs are not the most commonly used.

Adult stem cells are a positive alternative that does not interfere with the embryo controversy. Unfortunately, adult stem cells are rarer in mature tissues and isolating them is challenging [11]. The process of collecting hESCs is simpler compared to that of adult stem cell culturing, and as a result, hESCs continue to be experimented on. Eventually, there will be advancements in adult stem cell cultivation that may potentially eliminate the use of hESCs, but until then, hESCs have been deemed more efficient.

iPSCs are hypothesized to completely eliminate the use of hESCs, while compensating for the scarcity of adult stem cells [22]. However, as stated prior, it is unknown how iPSCs compare to hESCs. Researchers are unaware of their relative potential and whether they are safe for human transplantation [22]. As a result, hESCs are still favored for experimentation until advancements in iPSC research prove more efficient. Then, hESCs could possibly be a thing of the past.

The use of hESCs in research, no matter how beneficial it may be for finding cures, will always have some form of opposition. This results in a slower progression of finding cures, such as treatment for diabetes. The absence of public support negatively impacts the social sustainability of the technology. Regarding human embryos, what defines the beginning of human life is the obstacle between the present technology and further development [22]. However, stem cell engineering holds the potential to increase the quality of human life through diabetic treatment, which is a case for social sustainability. Unfortunately, the lack of advancements in adult stem cells and iPSCs make it difficult to immediately transition away from hESC. Stem cell research at this point in time is in a major predicament: if hESCs continue to be tested, many moral concerns will be brought up by opponents, but it is premature to transition to strictly using adult stem cells and iPSCs.

THE FUTURE OF T1D TREATMENT AND CHRONIC DISEASE

The technology of implementing stem cells to engineer pancreatic beta cells is relatively new but is perpetuating and advancing. Utilizing specific transcriptions factors to dedifferentiate somatic cells and differentiate hESCs, adult stem cells, and iPSCs leads to the development of insulin producing beta cells. These developed pancreatic cells are the necessary replacements to treat T1D. T1D is a chronic disease that lacks a definitive solution, but the advancements in stem cell engineering holds potential for an effective cure. With the variety of stem cells and transcription factors becoming much more prevalent, engineering cell replacements can lead to cures for a widespread of chronic illnesses, such as cancer, heart disease, and Alzheimer's. The sustainability of stem cell engineering is evident by present potential and future applications in progressing medical treatments for other chronic illnesses. Research and cures may take time due to opposition and unknowns of stem cells, however, the results hypothesized will be revolutionary in medicine.

SOURCES

[1] A. Menke, T. Orchard, et. al. “The Prevalence of Type 1 Diabetes in the United States.” Epidemiology, vol. 24. no. 5. September 2013. Accessed 2.27.2018. https://journals.lww.com/epidem/fulltext/2013/09000/The_Prevalence_of_Type_1_Diabetes_in_the_United.19.aspx

[2] “Type 1 diabetes.” Mayo Clinic. 8.7.2017. Accessed 1.13.2018. https://www.mayoclinic.org/diseases-conditions/type-1-diabetes/symptoms-causes/syc-20353011

[3] OpenStax, Anatomy & Physiology. OpenStax CNX. 2.26.2016. Accessed 2.24.2018. http://cnx.org/contents/14fb4ad7-39a1-4eee-ab6e-3ef2482e3e22@8.24

[4] D. M. Hillis, H. C. Heller, D. Sadava, M. V. Price. Principles of Life. Ed. 1. Sinauer Associates, Inc.: Sunderland, MA. 2012. pp. 216-218

[5] M. Skelin, M. Rupnik, A. Cenic. “Pancreatic Beta Cell Lines and their Applications in Diabetes Mellitus Research.” ALTEX. 5.17.2010. Accessed 1.12.2018. http://www.altex.ch/resources/altex_2010_2_105_113_Skelin2.pdf

[6] “Insulin, Medicines, & Other Diabetes Treatments.” National Institute of Diabetes and Digestive and Kidney Diseases. November 2016. Accessed 2.23.2018. https://www.niddk.nih.gov/health-information/diabetes/overview/insulin-medicines-treatments

[7] K.C. Claiborn, D.A. Stoffers. “Toward a cell-based cure for diabetes: advances in production and transplant of beta cells.” Mount Sinai Journal of Medicine. 2008. Accessed 2.20.2018. http://onlinelibrary.wiley.com/doi/10.1002/msj.20058/full

[8] C, Kent. “A New Way to Manage Type 1 Diabetes.” Review of Ophthalmology. 12.10.2016. Accessed 2.26.2018. https://www.reviewofophthalmology.com/article/a-new-way-to-manage-type-1-diabetes

[9] B. Hering, et. al. “Single-Donor, Marginal-Dose Islet Transplantation in Patients With Type 1 Diabetes.” The Jama Network. 2005. Accessed 2.19.2018. https://jamanetwork.com/journals/jama/fullarticle/200369

[10] “Sustainable Development Goal 3 Ensure healthy lives and promote well-being for all at all ages.” Sustainable

Development Knowledge Platform. Accessed 3.28.2018. https://sustainabledevelopment.un.org/sdg3
[11] "Stem Cell Basics I." National Institutes of Health. 2016. Accessed 1.13.2018. https://stemcells.nih.gov/info/basics/1.htm
[12] K. Takahashi, S. Yamanaka. "Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors." Cell, vol. 126, no. 4. August 2006. Accessed 2.9.2018. https://www.sciencedirect.com/science/article/pii/S0092867406009767?via%3Dihub
[13] "Gene Expression and Regulation." University of Leicester. Accessed 2.28.2018. https://www2.le.ac.uk/projects/vgec/highereducation/topics/geneexpression-regulation
[14] M. Korolija, M. P. Hadzija, M Hadzija. "Molecular mechanism in b-cell development: the role of Pdx1, Ngn3 and Pax4 proteins." Periodicum Biologorum, vol. 111, no. 1, 59–63, 2009. Accessed 2.20.2018. https://scholar.google.com/scholar_lookup?title=Molecular%20mechanism%20in%20beta-cell%20development%3A%20The%20role%20of%20Pdx1%2C%20Ngn3%20and%20Pax4%20proteins&author=Korolija%2CM&author=Hadzija%2CMP&author=Hadzija%2CM&journal=Period%20Biol&volume=111&pages=59-63&publication_year=2009
[15] D. Gerace, R. Martiniello-Wilks, B. A. O'Brien, A. M. Simpson. "The use of β-cell transcription factors in engineering artificial β cells from non-pancreatic tissue." Gene Therapy, vol. 22, no. 1. 1.2015. Accessed 1.11.2018 http://web.a.ebscohost.com/ehost/detail/detail?vid=6&sid=fdd0b3ad-2715-491a-989d-fb696418af40%40sessionmgr4009&bdata=JnNpdGU9ZWhvc3QtbGl2ZQ%3d%3d#db=aph&AN=100298167
[16] M. Sander, et. al. "Homeobox gene Nkx6.1 lies downstream of Nkx2.2 in the major pathway of β-cell formation in the pancreas." Development, vol. 127. 11.14.2000. Accessed 2.15.2018. http://dev.biologists.org/content/develop/127/24/5533.full.pdf
[17] "Microbiology." OpenStax. 2018. Accessed 2.28.2018. https://courses.lumenlearning.com/suny-microbiology/chapter/gene-therapy/
[18] "Pancreatic Islet Transplantation." National Institute of Diabetes and Digestive and Kidney Diseases. September 2013. Accessed 2.27.2018. https://www.niddk.nih.gov/health-information/diabetes/overview/insulin-medicines-treatments/pancreatic-islet-transplantation
[19] A. Trounson, et. al. "Clinical trials for stem cell therapies." BMC Medicine. 5.10.2011. Accessed 3.1.2018. https://bmcmedicine.biomedcentral.com/articles/10.1186/1741-7015-9-52#Tab2
[20] R. Millstein, et. al. "Insulin pumps: Beyond basal-bolus." Cleveland Clinic Journal of Medicine, vol. 82. no. 12:835-842. December 2015. Accessed 2.25.2018. https://www.mdedge.com/sites/default/files/issues/articles/Millstein_InsulinPumps.pdf
[21] S. Kesseli, K. Smith, T. Gardner. "Total Pancreatectomy with Islet Autologous Transplantation: The Cure for Chronic Pancreatitis?" Clinical and Translational Gastroenterology. vol. 6. Published 1.20.2015. Accessed 3.27.2018. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4418411/#bib34
[22] A. Siegel. "Ethics of Stem Cell Research." Stanford Encyclopedia of Philosophy. 1.28.2013. Accessed 1.13.2018. https://seop.illc.uva.nl/entries/stem-cells/

ADDITIONAL SOURCES

A. Niknamasl, S. Nasser Ostad, M. Soleimani, M. Azami, M. Kabir Salmani, N. Lotfibakhshaiesh, S. Ebrahimi-Barough, R. Karimi, R. Roozafzoon, J. Ai. "A new approach for pancreatic tissue engineering: human endometrial stem cells encapsulated in fibrin gel can differentiate to pancreatic islet beta-cell." Cell Biology International. vol. 38. no. 10. p. 1174-1182. 10.2014. Accessed 1.11.2018. http://rt4rf9qn2y.search.serialssolutions.com/?genre=article&title=Cell%20Biology%20International&atitle=A%20new%20approach%20for%20pancreatic%20tissue%20engineering%3A%20human%20endometrial%20stem%20cells%20encapsulated%20in%20fibrin%20gel%20can%20differentiate%20to%20pancreatic%20islet%20beta-cell.&author=Niknamasl%2C%20Azadeh&authors=Niknamasl%2C%20Azadeh%3BOstad%2C%20Seyed%20Nasser%3BSoleimani%2C%20Mansoureh%3BAzami%2C%20Mahmoud%3BSalmani%2C%20Maryam%20Kabir%3BLotfibakhshaiesh%2C%20Nasrin%3BEbrahimi-Barough%2C%20Somayeh%3BKarimi%2C%20Roya%3BRoozafzoon%2C%20Reza%3BAi%2C%20Jafar&date=20141001&volume=38&issue=10&spage=1174&issn=10656995
A. van Servellen, I. Oba. "Stem cell research: Trends in and perspectives on the evolving international landscape." Research Trends. 3.2014. Accessed 1.13.2018. https://www.researchtrends.com/issue-36-march-2014/stem-cell-research/
B. Newby, N. Terada, C. Mathews. "In search of a surrogate: engineering human beta cell lines for therapy." Science and Society. Vol. 25, No. 8. 8.2014. Accessed 1.11.2018. http://www.cell.com/trends/endocrinology-metabolism/pdf/S1043-2760(14)00099-X.pdf
M. Gunasekaran, N. Vachharajani, et. al. "Development of immune response to tissue-restricted self-antigens in simultaneous kidney-pancreas transplant recipients with acute rejection." Clinical Transplantation. vol. 31, no. 8. August 2017. Accessed 2.8.2018. http://onlinelibrary.wiley.com/doi/10.1111/ctr.13009/abstract
M. Korolija, M. P. Hadzija, M Hadzija. "Molecular mechanism in b-cell development: the role of Pdx1, Ngn3 and

Pax4 proteins." Periodicum Biologorum, vol. 111, no. 1, 59–63, 2009. Accessed 2.20.2018. https://scholar.google.com/scholar_lookup?title=Molecular%20mechanism%20in%20beta-cell%20development%3A%20The%20role%20of%20Pdx1%2C%20Ngn3%20and%20Pax4%20proteins&author=Korolija%2CM&author=Hadzija%2CMP&author=Hadzija%2CM&journal=Period%20Biol&volume=111&pages=59-63&publication_year=2009

R. Horch, L. Popescu, C. Vacanti, G. Maio. "Ethical Issues in Cellular and Molecular Medicine and Tissue Engineering." National Institutes of Health. 8.9.2008. Accessed 1.14.2018. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4506149/

S. Shin, C. H. Jung, et. al. "Long-term effects of pancreas transplant alone on nephropathy in type 1 diabetic patients with optimal renal function." Plos. 1.29.2018. Accessed 2.1.2018. http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0191421

"What Biomedical Engineers Do." Occupational Outlook Handbook. 10.24.2017. Accessed 1.12.2018. https://www.bls.gov/ooh/architecture-and-engineering/biomedical-engineers.htm#tab-2

ACKNOWLEDGEMENTS

We would like to thank our writing instructor, Nancy Koerbel, our co-chair, Rahul Ramanathan, and our section chair, Morgan Austin for guiding us to a more successful paper. We would especially like to thank Austin Sims for bringing together our most efficient team. And of course, our families, because of their dedicated support.