THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF BIOMEDICAL ENGINEERING

Differentiation of iPSC-Derived Beta Cells via the Use of Modified mRNA

RACHEL HOENISCH
SPRING 2022

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Biomedical Engineering
with honors in Biomedical Engineering

Reviewed and approved* by the following:

Xiaojun Lance Lian
Associate Professor of Biomedical Engineering
Thesis Supervisor

Nanyin Zhang
Professor of Biomedical Engineering and Electrical Engineering
Honors Adviser

* Electronic approvals are on file.

ABSTRACT

Type 1 diabetes is a disease that impacts millions of patients around the world. Because of the severity of the disease and the multitude of associated conditions that can develop if it is not properly controlled, improving treatment options for people with Type 1 diabetes is of extreme importance. Stem cells offer one route to accomplish this, but at the moment, many methods of differentiating induced pluripotent stem cells (iPSCs) into pancreatic beta cells are not as efficient as they need to be for large-scale production of beta cells. Modified mRNA has been used in the differentiation protocols of other cell types, so it has potential to be used in the differentiation of pancreatic beta cells as well. This study will evaluate the current feasibility of using modRNA to facilitate the differentiation of iPSCs into pancreatic beta cells through experimentation and examination of the literature. The use of lipofection as a transfection method will be examined using the gene PDX1 as an example, and the success of each transfection factor will be assessed using c-peptide staining.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

I would like to thank Dr. Lian for accepting me into his lab in the beginning of my junior year, at a time when so much was uncertain. I'd also like to thank Yuqian Jiang for training me and allowing me to assist with her project over the summer, and Alessandro Howells for training me on modRNA, cell culture, and everything else he has done for this project. Finally, I'd like to thank my friends and family for being with me every step of the way.

Chapter 1

Background

Overview

The rising prevalence of Type 1 diabetes, along with the array of complications that can be caused if the disease is poorly managed, makes it more important than ever for new treatment approaches to be developed. Current treatments are often effective, but can be costly and physically painful for the patient. Induced pluripotent stem cells are a promising possibility, but if they are to be feasible as a commercially available treatment for Type 1 diabetes, the process of differentiating them into pancreatic beta cells must be improved. Using modified mRNA to facilitate differentiation could make this process easier, safer, and more efficient, making it much simpler for labs to produce sizable quantities of pancreatic beta cells that could be used for either research or treatment.

Type 1 Diabetes

Type 1 diabetes, also known as diabetes mellitus, is characterized by an inability of the pancreas to regulate the blood glucose levels of a patient. While Type 1 diabetes is far less prevalent than Type 2 diabetes, comprising only 5-10% of known diabetes cases as of 2006, it is a major public health concern both in the United States and worldwide [1]. Additionally, it appears that prevalence of Type 1 diabetes is increasing. In the United States, the prevalence of Type 1 diabetes in children is estimated to have increased roughly 45% from 2001 to 2017, while

worldwide prevalence was estimated to be increasing by 3% each year in 1999 [2] [3]. Several hypothesis have been put forward speculating on the reasons behind this steady increase, but as of the writing of this paper, no concrete cause has been identified [4].

Despite this, much progress has been made in determining the mechanisms behind the onset of Type 1 diabetes. The disease begins when the body mounts an autoimmune attack on pancreatic beta cell islets [1]. Leukocytes invade the islets and prompt T cells to begin the destruction of beta cells, resulting in a decline in the amount of insulin being produced. In most—but not all—cases, this autoimmune response continues until the pancreas is rendered completely nonfunctional, unable to produce insulin or glucagon in response to blood glucose fluctuations [5]. It is currently thought that the autoimmune response may be brought on by exposure to certain viruses, environmental factors, or vitamin D. Additionally, certain genetic markers have been identified with increased susceptibility to the development of Type 1 diabetes, but some argue that the influence of genetics on susceptibility to the disease is decreasing with time [1] [6].

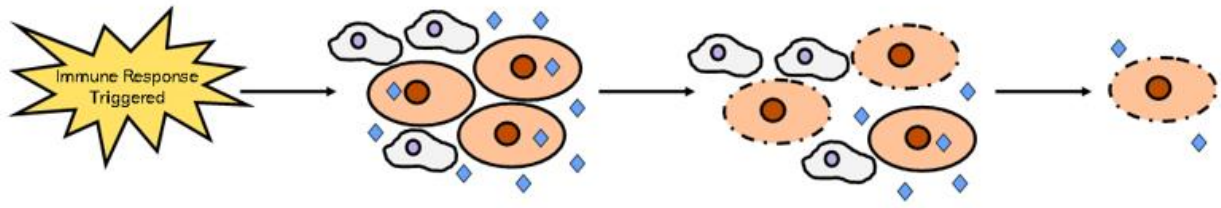

Figure 1. Progression of immune attack during the onset of Type 1 diabetes.

This increased understanding of the disease's onset has prompted some efforts to prevent the autoimmune response from occurring in the first place. In 2004, a trial of nicotinamide for this purpose took place in individuals identified as being at risk for developing Type 1 diabetes, but it was found to be ineffective [7]. Due to failures such as these, focus has shifted somewhat from prevention to increasing the standard of care for current and future patients.

If not properly treated, Type 1 diabetes can lead to the development of additional medical conditions. The risk to the kidneys is especially apparent, with 50-70% of patients developing kidney disease over the course of their lives. Hyperglycemia was identified as a possible contributor to this observation [8]. Kidney disease has also been found to be among the primary indicators of mortality in Type 1 diabetes patients, so if an increase in treatment regimens can lower patients' risk of kidney issues, many lives may be saved [9]. Also of concern are the increasing prevalence of obesity in untreated Type 1 diabetes patients and an increased susceptibility to infections associated with the systemic effects of long-term hyperglycemia [10] [11]. From this, it can be concluded that high-quality treatment is of paramount importance in improving the outcome of diabetes patients.

Current Standard of Care

There exists a wide range of treatment options for Type 1 diabetes. After the initial diagnosis is made and the patient is discharged from inpatient care, glucose monitoring and the administration of insulin becomes a daily routine. For many patients over the past couple of decades, this has meant finger-pricking and insulin injections [12]. However, the advent of the insulin pump and continuous glucose monitor (CGM) has greatly increased the standard of care. When used together, these two devices form a closed-loop system, often colloquially referred to as an "artificial pancreas". These closed-loop systems have been observed to improve a patient's ability to remain within a set range of acceptable blood glucose values, but it may not be as

effective in children, who might not use the system to its full potential due to psychological and social factors [13] [14] [15].

Table 1. Current Methods of Treatment for Type 1 Diabetes

Name of Treatment	Pros of Treatment	Cons of Treatment
Insulin Injections	+ Less expensive	- Requires calculations - Many painful needle pokes - Insulin must be carried to each meal
Insulin Pen	+ Simple to use + Doses are pre-measured	- Many painful needle pokes - Limited number of doses
Insulin Pump	+ Doses are automatically measured + Calculations are automatically performed + One injection every few days + Ability to administer a basal dose of insulin	- Expensive - Can be difficult to learn how to use - Chance of occlusions in tube or other hardware/software malfunctions
Continuous Glucose Monitor	+ Fewer finger pricks necessary + Allows for tighter control of blood glucose levels	- Expensive - Not always accurate
Closed-Loop System	+ Requires almost no user input once configured + Fewer finger pricks and injections necessary	- Expensive - Not compatible with all pump and CGM types

While these innovations have certainly made an impact in the lives of Type 1 diabetes patients, it is clear that there is still a long way to go before they can take anything other than an active role in managing their disease. Because of this, many researchers are adopting a stem cell-oriented approach to diabetes treatment.

Stem Cells for the Treatment of Type 1 Diabetes

The concept of using stem cells for the treatment of diseases such as Type 1 diabetes is not new, but in recent times, technology has progressed to the point that what was once impossible has become commonplace. In 2009, Maehr *et al.* produced induced pluripotent stem cells from Type 1 diabetes patients using transcription factors SOX2, OCT4, and KLF4 and demonstrated that these cells could differentiate into pancreatic beta-like cells [16]. Since then, stem cell-derived pancreatic beta cells have been transplanted into mice and rats in order to determine their therapeutic potential. In many cases, the findings have been encouraging. Nuclear transfer embryonic stem cells have been observed to secrete insulin in response to fluctuations in blood glucose when implanted in mice, and adipose mesenchymal stem cells were shown to have a therapeutic effect when transplanted into rats with induced Type 1 diabetes [17] [18].

Induced pluripotent stem cells (iPSCs), however, are one of the most promising candidates for large-scale treatment of Type 1 diabetes. Because iPSCs can be generated from individual patients, the problems posed by immune rejection and a limited supply of other types of stem cells can be alleviated [19]. iPSC-derived human islet-like organoids have also been observed to function when implanted in mice *and* evade immune detection [20]. Because of this potential, it is critical to develop efficient, simple protocols to drive the differentiation of iPSCs into pancreatic beta cells. Many current protocols do not produce pancreatic beta cells that are capable of generating insulin, and while Wnt-inhibition can improve outcomes, it is still far from perfect [21].

ModRNA

Modified RNA (modRNA) is a promising new tool for the differentiation of iPSCs. In this technique, genes coding for certain transcription factors can be directly inserted into iPSCs via lipofection with lab-produced strands of modRNA. Once inside the cell, the modRNA is translated by the cell machinery and the transcription factors begin modulating the expression of genes related to cell fate. If the correct combination of transcription factors is used, the iPSCs differentiate into the desired mature cell type without a need for the complex protocols currently used. This method dramatically cuts down on the labor-intensive steps of treating the iPSCs with multiple different compounds while increasing the efficiency of differentiation [22].

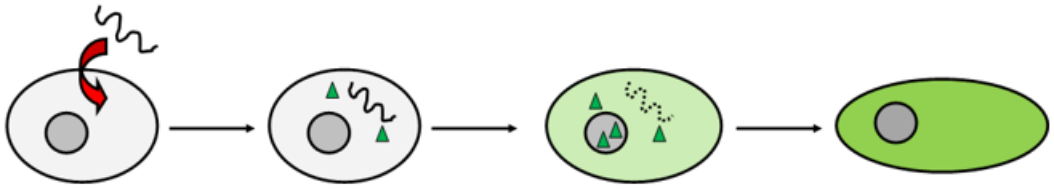

Figure 2. Mechanism behind the modRNA stem cell differentiation method.

ModRNA has already been successfully used for the differentiation of several different cell types. Through this method, human pluripotent stem cells (hPSCs) have been differentiated into kidney tissue and iPSCs have been differentiated into endothelial cells [23] [24]. However, in order for modRNA to be effectively used, the transcription factors necessary for the differentiation into a certain cell type must first be identified. Oftentimes, several transcription factors are needed, and the inclusion of an unnecessary transcription factor can actually inhibit the pathways necessary for differentiation.

Due to the vast number of genes encoding for transcription factors in the human genome, it can be extremely difficult to identify a group of transcription factors that will lead to

differentiation into a desired cell type. At this time, these transcription factors remain unidentified for pancreatic beta cells.

Chapter 2

Purpose

Limitations of Current Differentiation Techniques

As mentioned in the previous chapter, it is currently possible to produce pancreatic beta cells from iPSCs using existing differentiation protocols. However, many of these protocols would not be practical for large-scale use for a variety of reasons. First, the average protocol consists of a several step long process involving the addition of many compounds meant to activate and inhibit key pathways within the cell. These compounds must be manually added to a culture vessel at key intervals, which increases the amount of labor that goes into the production of a batch of differentiated cells and introduces the possibility of contamination from repeated opening and closing of the culture vessel. Another limiting factor of the practicality of existing protocols is their low efficiency. When a differentiation protocol is applied to iPSCs, not all of the iPSCs undergo full differentiation, and some do not differentiate at all. This results in a final culture that is composed of a mixture of differentiated and undifferentiated cells. The undifferentiated cells must then be separated out from the mature, differentiated cells, which further increases the amount of labor involved in the entire process.

Reason for Using modRNA for Differentiation

By utilizing modRNA for iPSC differentiation, many of the drawbacks listed in the previous section are alleviated or removed entirely. The ideal modRNA protocol would require only the addition of the modRNA to the cells—along with proper cell culture techniques—for

differentiation into pancreatic beta cells to take place. This would cut down on a great deal of complexity and decrease the amount of materials required. Because modRNA has also been observed to be more efficient than other methods of iPSC differentiation, a greater number of mature pancreatic beta cells can be produced using the same number of starting iPSCs, reducing the cost of the process and making it more feasible to produce large batches of cells. The increased efficiency of the modRNA method also increases the safety of implanted iPSC-derived pancreatic beta cells. This is because undifferentiated iPSCs, if implanted into an animal, will continue to grow and divide as if in a culture vessel, with no regard to the surrounding tissue. The resulting growth, called a teratoma, is essentially a tumor [25]. Like a tumor, if dividing iPSCs break off from the main growth and travel elsewhere in the body, the affliction can spread throughout an organism. Efforts are made to separate differentiated cells from undifferentiated iPSCs prior to implantation, but the increased differentiation efficiency conferred by modRNA could decrease the chance of any rogue undifferentiated iPSCs making it into a test animal or patient.

Overall, the modRNA approach to iPSC differentiation has the potential to be simpler, cheaper, safer, and more efficient than existing protocols. If the stem cell approach to treating Type 1 diabetes does become feasible in the coming years or decades, it is critically important that supply will be able to meet demand, and the modRNA approach could help make this possible.

Experimental Structure

In order to determine the composition of modRNA that will facilitate the differentiation of iPSCs into pancreatic beta cells, key transcription factors must first be identified. It would be ideal to include dozens or hundreds of transcription factors in this process so any relevant transcription factors are not missed, but for practicality's sake, the number of transcription factors included in the study must be limited. Ultimately, eleven transcription factors were chosen. Any combination of the included transcription factors could be required for differentiation; one, two, or even all eleven could be necessary for the production of mature insulin-producing beta cells. Likewise, it is also possible that *none* of the included transcription factors influence differentiation when introduced to iPSCs through modRNA. For this reason, it is important to carefully evaluate each possible combination of transcription factors in order to determine if any are having an effect.

Such a process is incredibly time consuming, but by identifying any critical transcription factors early on, the experiment can be streamlined. In order to achieve this, each of the eleven genes were individually transfected into iPSCs. After time was allowed for preliminary differentiation/maturation to occur, the cells were stained for c-peptide to determine whether any meaningful differentiation into pancreatic beta cells had taken place. If any of the cells begin differentiating, it can be concluded that the transcription factor they were transfected with is worth including in the combination that is theoretically necessary for full differentiation to take place.

Additionally, it must be determined whether the cells were successfully transfected with the modRNA bearing the transcription factors before any further experimentation can take place. Effectiveness of modRNA transection was assessed by utilization of GFP staining 24 hours post-

lipofection. For the purposes of this study, PDX1 was used as a sample transcription factor to determine whether the modRNA had successfully entered the cells.

Chapter 3

Methods and Materials

Targeted Transcription Factors

The transcription factors targeted in this study were identified by their involvement in cell differentiation, pancreatic development, Type 1 diabetes onset, and insulin regulation. Table X shows the eleven transcription factors that were cloned into plasmids in order to facilitate the production of modRNA, as well as their observed or predicted functions according to NCBI [26].

Table 2. Transcription Factors Included in Study

Transcription Factor	Role
SIX2	May be linked to eye and limb development
ISL1	Plays a role in development of pancreatic cell lineages
FOXA1	Activator for liver transcripts, involved in pancreatic cell differentiation in mice
FOXA2	May be linked to onset of Type 1 diabetes
NKX2.2	Nuclear transcription factor
NKX6.2	May be linked to cell differentiation
NEUROD1	Regulates the insulin gene
PAX6	Plays a role in development of eye and neural tissue
NGN3	Linked to neurogenesis
MAFA	Involved in the regulation of the insulin gene in pancreatic beta cells
PDX1	Linked to pancreas development and regulation of the insulin gene;

Sequencing Transcription Factors

Genes received from Germany were sequenced using the Penn State Genomics Core Facility. The resulting sequence was compared with the gene sequence on file in NCBI in order to ensure that no mutations were present.

Backbone Digestion

5MCS3 was used as the vector plasmid for this experiment. EcoRI and SpcI were used as restriction enzymes. To perform backbone digestion, 2 ug (8.29 uL) of 5MCS3, 2 uL of EcoRI, 2 uL of SpcI, 5 uL of CutSmart buffer, and 32.71 uL of water were added to a PCR tube and incubated for 1 hour at 37°C. After incubation, gel electrophoresis and UV imaging were used to locate and excise the backbone band. The ZymoClean gel DNA recovery kit was used to retrieve the DNA, and the concentration of the backbone was verified using a Nanodrop machine.

Production of Gene Inserts

For each insert, Q5 High-Fidelity DNA polymerase was used for amplification. 10 uL Q5 Reaction Buffer (5X), 1 uL dNTP (10 mM), 5 uL primer mix (5 uM), 1 uL template DNA (10 ng/uL), 10 uL GC enhancer (5X), 0.5 uL Q5 polymerase, and 22.5 uL of water were added to a PCR tube and placed into a thermocycler. The annealing temperature varied with the primers used, and the elongation time was calculated by using a 30s per kB protocol. Gel electrophoresis was used to excise the amplified insert, and the ZymoClean gel DNA recovery kit was used to retrieve the insert from the gel.

Ligation of the DNA insert and the backbone vector was accomplished using the In-Fusion kit. The amount of insert and vector to be used in the kit was calculated for each insert, and the calculated amount was added to 2 uL of enzyme premix and water to a total volume of 10 uL. This mixture was incubated at 50°C for 15 minutes.

Production of the resulting plasmid was achieved via *E. coli* culturing. 5 uL of the plasmid from the previous step was transformed to Thermo Stbl3 *E. coli*, and after the *E. coli* were allowed to grow overnight, three colonies were chosen for sequencing to ensure that the plasmid had been properly transformed.

modRNA Production

The 5MSC3 plasmid was diluted to 1 ng/uL using water. Then, 10 uL of the plasmid solution (1 ng/uL), 10 uL of primer mix (1 uM), and 20 uL Q5 High-Fidelity 2X Master Mix were added to a PCR tube. This step was duplicated to make another tube, and the two samples were placed in a thermocycler. After thermocycling, gel electrophoresis and UV visualization were used to ensure that PCR proceeded correctly. The contents of both tubes and 6 uL of Dpn1 were combined into a single tube, which was incubated at 37°C for 20 minutes. Zymo DNA Clean and Concentrator – 5 was used to purify the resulting product. Omega buffer was used to adjust the final concentration to 100 ng/uL.

A custom NTP solution was made by combining 5 uL ARCA, 1.8 uL GTP, 5.4 uL ATP, 5.4 uL CTP, 1.35 uL 1-M-psuedo-UTP, and 1.05 uL of water. Then, 16 uL of this custom NTP solution was added to 16 uL PCR product, 4 uL MEGAscript 10X buffer, and 4 uL MEGAscript T7 enzyme. The resulting mixture was incubated at 37°C for 4 hours. After incubation was complete, 2 uL DNase was added and the mixture was incubated at 37°C for 15 minutes.

Lipofection and Cell Culture

Culturing of iPSCs was performed according to current protocols. Once these cells reached roughly 30% confluency, lipofection was performed in order to introduce the modRNA into the iPSCs. It should be noted that all volumes noted in this section assume a 6-well plate; for volumes corresponding to 12 and 24-well plates, values should be doubled and quadrupled, respectively. First, fresh mTeSR was used to replace the culture's previous media. Then, two tubes were prepared. In the first tube, 100 uL of Opti-MEM 1 medium was combined with 4 uL of Lipofectamine Stem reagent. In the second tube, 100 uL of Opti-MEM 1 medium was combined with 1000 ng of the desired modRNA. The contents of Tube 2 were added to Tube 1 and mixed. Then, Tube 1 was incubated at room temperature for 10 minutes. 200 uL of the resulting mixture were added to each well of a 6-well plate, which was then placed in an incubator overnight at 37°C with 5% CO_2 overnight. After this incubation, 0.5 mL of mTeSR medium should be added to each well.

Data Analysis

In order to ensure that lipofection was successful, imaging of GFP was performed in a sample population of PDX1-transfected cells. Cells were allowed to culture for 24 hours after lipofection, then images were taken and analyzed to determine whether a sizable portion of the cells were expressing GFP. GFP imaging was also performed for the other ten transcription factors using an identical method.

A similar process was used to assess the role of the eleven targeted transcription factors in pancreatic beta cell differentiation. Instead of GFP, c-peptide was used to determine whether

any of the iPSCs were showing signs of differentiation. The amount of fluorescence in the images was quantified using ImageJ. For each image, the area above the scale bar was selected using the Rectangle tool, and the Measure tool was used to find the mean brightness of each image. The scale bar was excluded from this analysis due to the fact that the white font would artificially increase the mean brightness of each image. The mean brightness of each transcription factor was then divided by the mean brightness of the wild type image in order to find the relative brightness compared to wild type.

Chapter 4

Results

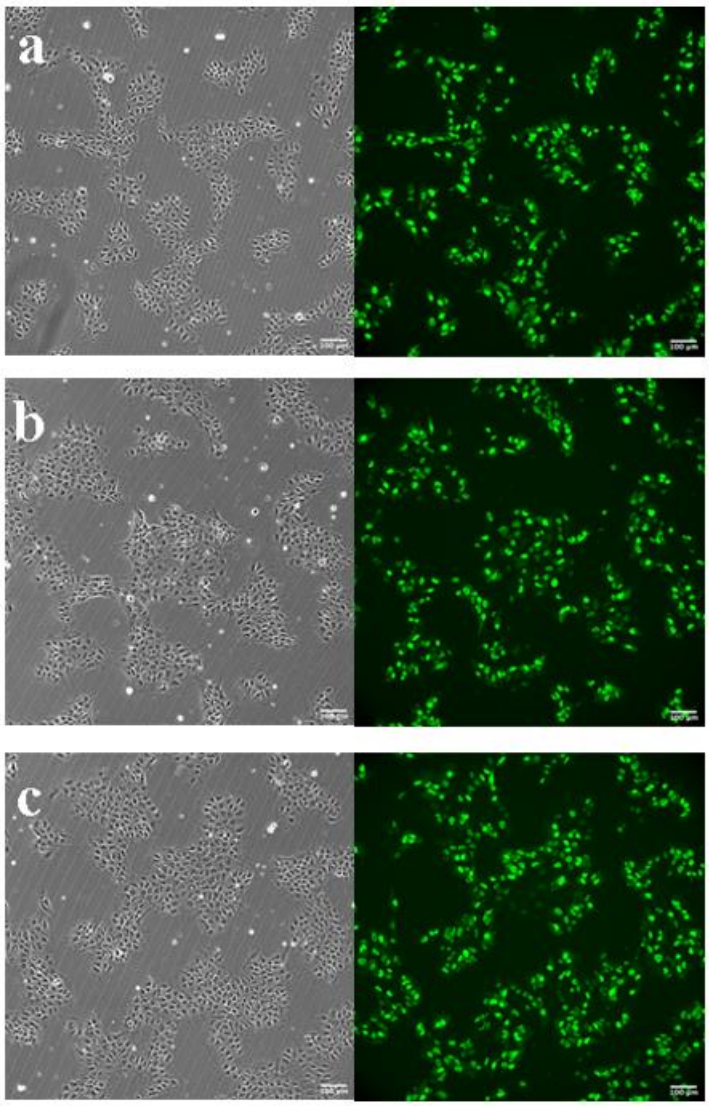

Figure 3. Imaging of PDX1-transfected iPSCs

In order to determine whether the transfection of modRNA into iPSCs was successful, the iPSCs were imaged 24 hours after lipofection took place [27]. Figure 3 shows the results of this imaging for three different sites chosen in the cell culture wells, with each panel corresponding to a site. The images on the left of each panel depict images taken during bright field

microscopy, and the images on the right of each panel show the presence of GFP in each site. The bright field images are rather similar between sites, and appear to show the aggregation of the iPSCs into loose clumps. Likewise, there are few observable differences between the GFP images of each site, which, when overlaid with the respective bright field images, show that GFP is being expressed by the majority of the iPSCs pictured.

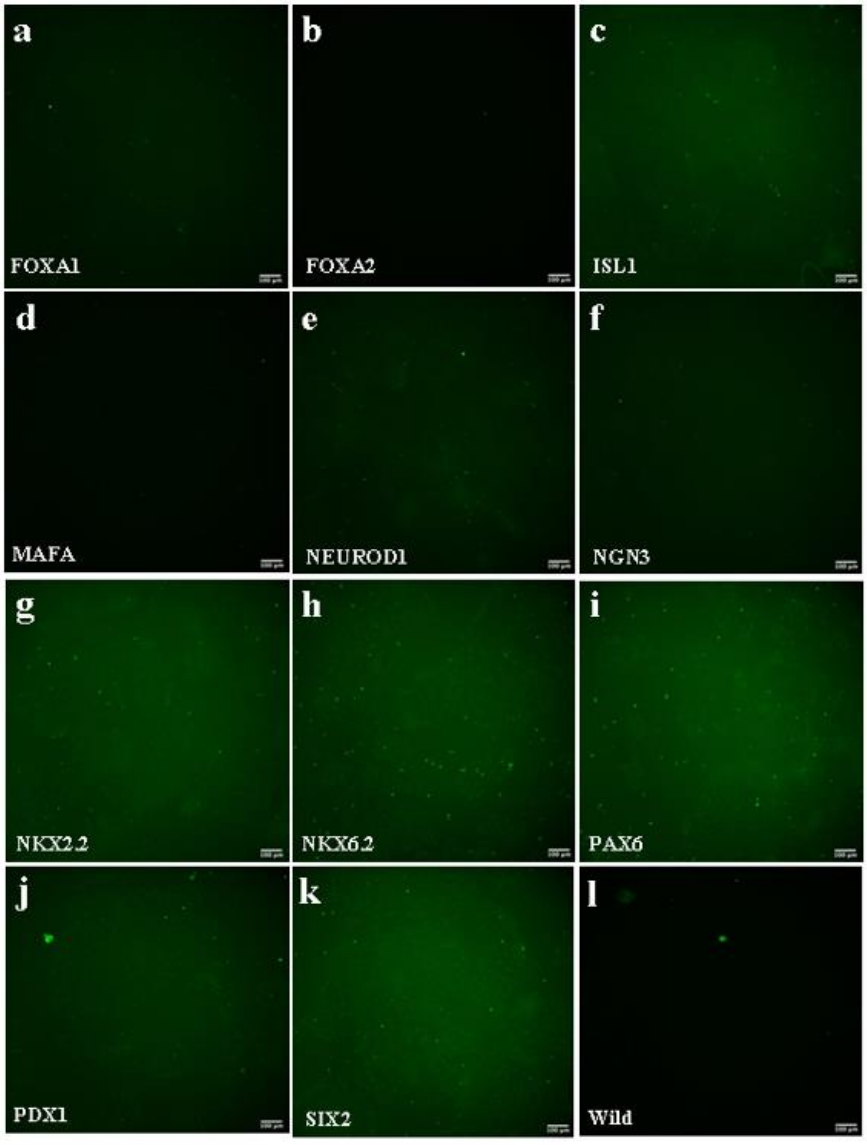

Figure 4. C-Peptide Staining of iPSCs

Preliminary assessment of the impact each transcription factor had on pancreatic beta cell differentiation was carried out by staining for c-peptide [27]. Figure 4 shows the results of this staining for each of the eleven transcription factors included in the study, as well as the result of staining in a wild-type population of iPSCs that were not transfected with modRNA. Observation of panels b, d, and f, which correspond to FOXA2, MAFA, and NGN3, show little to no expression of c-peptide. Panels a, e, and l, which correspond to FOXA1, NEUROD1, and wild type, show trace expression of c-peptide as a single dot of green fluorescence. Similarly, panel j, which corresponds to PDX1, also shows a single dot of fluorescence, but the dot is larger, appearing to be an aggregate of at least a few cells. This may suggest a slightly greater degree of c-peptide expression than what was observed in panels a, e, and l. Panels c, g, h, i, and k, which correspond to ISL1, NKX2.2, NKX6.2, PAX6, and SIX2, appear to show low to moderate levels of c-peptide expression in the form of a cloudy haze of green fluorescence accompanied by scattered dots of brighter fluorescence.

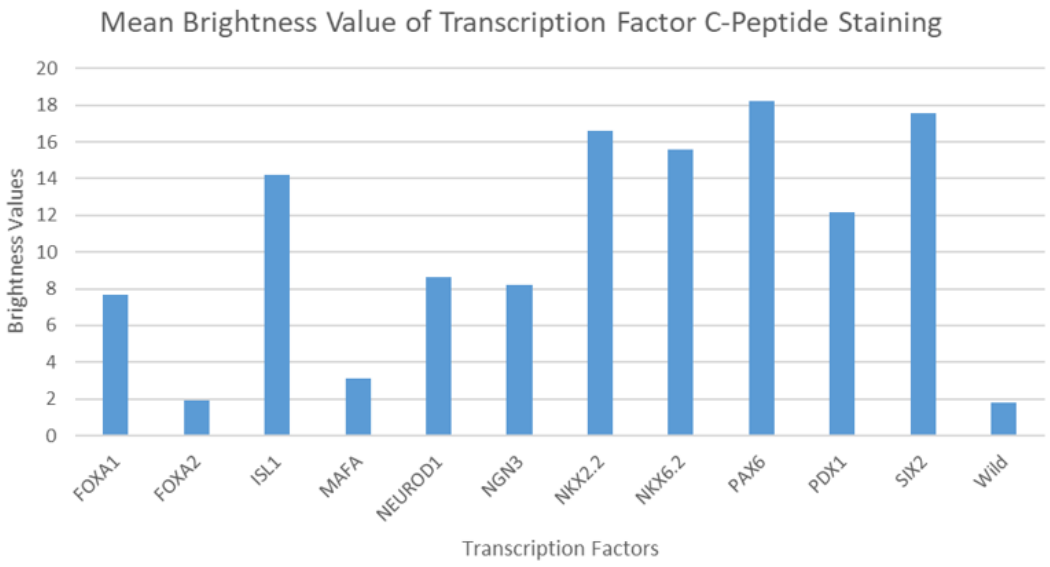

Figure 5. Graph of Mean Brightness Values

Quantification of the c-peptide staining images shown in Figure 4 was accomplished using ImageJ. Figure 5 shows the results of an image brightness measurement performed for each transcription factor, which are similar but not identical to the results shown in Figure 4. FOXA2, MAFA, and wild type iPSCs had the lowest brightness values, while ISL1, NKX2.2, NKX6.2, PAX6, and SIX2 had the highest brightness values.

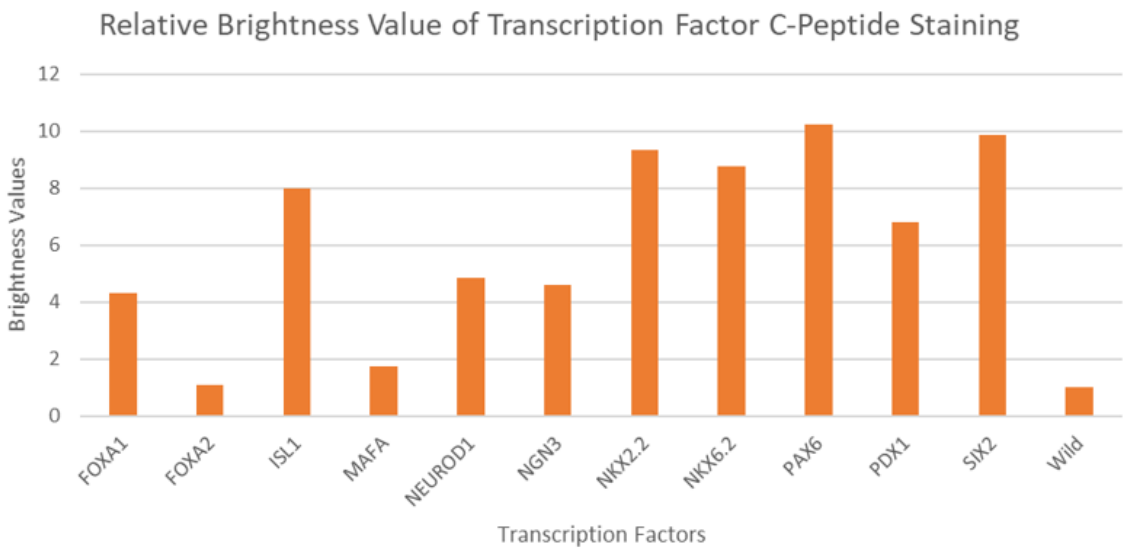

Figure 6. Graph of Relative Brightness Values

Table 3. ImageJ Brightness Value Data

Gene	Mean Brightness Value	Relative Brightness Value
FOXA1	7.698	4.327
FOXA2	1.929	1.084
ISL1	14.221	7.994
MAFA	3.107	1.746
NEUROD1	8.609	4.839
NGN3	8.195	4.607
NKX2.2	16.59	9.325
NKX6.2	15.608	8.773
PAX6	18.212	10.237
PDX1	12.138	6.823
SIX2	17.579	9.881
Wild	1.779	1.000

The relative brightness of each transcription factor as compared to the wild type image is shown in Figure 6. PAX6 and SIX2 had the highest relative brightness values at about ten times the brightness of the wild type. FOXA2, on the other hand, was roughly equal to the brightness

of the wild type. The raw data for each transcription factor's mean and relative brightness value is shown in Table 4.

Chapter 5

Discussion

Lipofection as a Transfection Method

To determine whether transfection of modRNA into iPSCs was successful, immunostaining of transfected cells was carried out 24 hours after transfection. The modRNA to be inserted into the cells contained the gene coding for PDX1 as well as GFP, which would produce fluorescence in successfully transfected cells. In Figure 3, abundant expression of GFP—as shown by the green areas in the image—suggests that the lipofection procedure was, in fact, successful. When compared with the bright field images also included in Figure 3, it appears that nearly all visible cells at each imaged site expressed GFP, so it follows that the PDX1 was also successfully delivered.

Lipofection has been used previously to transfect siRNA into target cells, but modRNA is much larger than siRNA, which led to doubts over whether lipofection would be capable of carrying out efficient transfection. However, lipid nanoparticles have been shown to be capable of delivering modRNA to an area of cardiac infarction in living mice [28]. It is worth mentioning that *in vivo* use in mice is quite different from *in vitro* transfection of stem cells. The former involves the implantation of lipid nanoparticles into a living animal, while the latter involves rapid delivery of RNA into cells in culture. Environmental factors between these two scenarios are radically different, so it is necessary to evaluate the performance of lipofection in scenarios more akin to what would be present in stem cell research.

As discussed above, Figure 3 serves as such an indication, showing plentiful expression of GFP that was added to iPSCs via lipofection. This is far from an isolated result. PCR has been used to quantify the successful lipofection of GFP into human mesenchymal stem cells, and more recently, lipofection was successfully used to deliver modRNA to human cell lines [29] [30]. From these examples in the literature, along with the data gathered in Figure 3, it can be concluded that lipofection is an effective tool for transfection of modRNA into stem cells, which is achieved without the safety questioned posed by viral carrier methods of transfection [29].

C-Peptide Expression of iPSCs after Transcription Factor Delivery

C-peptide is commonly produced by pancreatic beta cells at roughly equal levels as insulin, so it is often used as a marker for Type 1 diabetes. If a patient's blood or urine test yields low levels of c-peptide, this is an indication that their levels of insulin are also abnormally low [31]. It follows, then, that c-peptide is also an effective marker for the differentiation of stem cells into insulin-producing pancreatic beta cells. After iPSCs were transfected with one of the eleven transcription factors included in the study, immunostaining for c-peptide was performed in order to determine whether any of the cells had undergone a substantial degree of differentiation into pancreatic beta cells. As mentioned in the results section and shown in Figures 3, 4, and 5, FOXA2 and MAFA yielded the least fluorescence, which indicates that little to no differentiation took place when only these transcription factors were present. In fact, the brightness value of FOXA2, found in Table 4, was only 1.084 times that of the wild type image, suggesting that treatment with FOXA2 yields roughly the same results as no treatment at all.

This does not mean that FOXA2 and MAFA play no role in pancreatic beta cell differentiation, but it does indicate that either of these transcription factors on their own are not enough to facilitate differentiation.

From a qualitative analysis of the images shown in Figure 3, it appears that NKX2.2, NKX6.2, PAX6, and SIX2 were all roughly equal in terms of c-peptide expression, and thus equally effective in facilitating differentiation. Quantitative analysis of image brightness as shown in Table 4, however, suggests that PAX6 was the most successful transcription factor, with a mean brightness value of 18.212 and a relative brightness value of 10.237. Therefore, in this particular study, transfection with PAX6 resulted in the highest amount of expressed c-peptide. Any future combination of transcription factors, then, would likely benefit from including PAX6. NKX2.2, NKX6.2, and SIX2 are also relatively viable candidates, with relative brightness values of 9.325, 8.773, and 9.881, respectively.

However, it should be noted that none of the individual transcription factors yielded a particularly impressive differentiation efficiency. Even the images with the highest relative brightness are rather dim, with the majority of the cells present failing to express c-peptide. This observation can be tempered by the fact that it was extremely unlikely for a single transcription factor to result in highly efficient differentiation; the expectation going into this study was that a *combination* of transcription factors would be necessary. With that in mind, these results should be taken not as an endpoint, but the beginning of a larger investigation.

Possible Influence of Targeted Transcription Factors on Differentiation

While a short summary of each transcription factor's function was given in Table 2, the reality is that transcription factors often perform several different tasks, some of which are still unverified or unknown. It is appropriate, then, to apply what is currently understood about these transcription factors to the results yielded in this experiment in order to speculate about the possible cause of differing levels of c-peptide expression.

FOXA2 may have shown the least c-peptide expression of all eleven studied transcription factors, but this does not mean it plays no role in pancreatic beta cell differentiation. In fact, it—along with FOXA1—has been found to be critically important in pancreas development as an enhancer of PDX1 [32]. Individually, it did not result in a large amount of differentiation in the treated iPSCs, but if it were to be combined with FOXA1, perhaps more differentiation would have been observed. FOXA1 was also observed to result in rather low c-peptide expression, though not quite as low as what was seen in the FOXA2-transfected cells. It may also be beneficial to combine these two transcription factors with PDX1, the transcription factor they regulate.

Though seemingly slightly more effective than FOXA2, MAFA was the transcription factor that produced the second least amount of c-peptide in iPSCs. MAFA is involved in the development of the pancreas and the activation of the insulin gene, but timing has been found to be critically important in ensuring that its expression positively impacts differentiation from pancreatic progenitors into beta cells [33]. Therefore, timing might explain the relatively small impact MAFA had on facilitating c-peptide expression in the treated population of cells. MAFA has also been observed to have strong interactions with PDX1 and NGN3, which could also factor into its lackluster importance in this experiment [33]. Similarly to the situation described

with FOXA2 and FOXA1, it may be beneficial to examine the effect of a combination of MAFA, PDX1, and NGN3 on differentiation.

The same can be said for NGN3. This transcription factor has been generally linked with development of the pancreas, but despite this importance, it is not entirely clear if it is necessary for differentiation into pancreatic beta cells [33]. Alone, it appeared to result in very little production of c-peptide, but combining it with MAFA and PDX1 could improve the outcome of differentiation.

PDX1 was observed to result in more fluorescence—and thus more c-peptide expression—than either MAFA or PDX1, but it should be noted that a small cluster of strong fluorescence in the image may have played a factor in this. However, it is known as an extremely important factor in the proper function of pancreatic beta cells due to its role as a promoter of the insulin gene. Even after differentiation and maturation of beta cells, continued function of PDX1 is necessary to maintain a proper production of insulin [33]. Once again, despite this importance, it is likely that PDX1 needs to be combined with other transcription factors to result in efficient differentiation of iPSCs into pancreatic beta cells. Considering its importance in beta cell function, it might also be prudent to include it in *any* future combination of transcription factors included in modRNA transfection.

ISL1, NKX2.2, and NKX6.2 were all on the more effective end in terms of how much c-peptide expression was observed post-transfection. ISL1 is linked to the proliferation of pancreatic islets [34], NKX2.2 is involved in a repression complex that both promotes differentiation into beta cells and prevents differentiation into alpha cells [35], and NKX6.2 has been observed to be involved in determining whether a pancreatic cell becomes an alpha cell or a beta cell [36]. While relatively similar levels of c-peptide were expressed by the iPSCs

transfected with each of these three transcription factors, it does not appear that any of them are individually sufficient to facilitate effective, large-scale differentiation. Combining the three would be an interesting experiment, but they do not appear to be as functionally intertwined as MAFA, PDX1, and NGN3, or even FOXA1, FOXA2, and PDX1.

NEUROD1 did not result in the production of as much c-peptide as many of the other transcription factors included in this study, despite its strong association with the insulin gene and the fact that its absence is linked to the development of diabetes [37]. Perhaps, like MAFA, timing played a role in this result, or perhaps this transcription factor simply needs to be paired with transcription factors that were found to be more effective. It is unclear whether the inclusion of this transcription factor would be strictly necessary for a successful modRNA treatment, since several of the other transcription factors tested are also linked to regulation of the insulin gene. However, on an individual basis, it was observed to result in the production of more c-peptide than either FOXA1 or MAFA, the two lowest-performing transcription factors in the study.

Of the eleven transcription factors included in this experiment, PAX6 and SIX2 were the highest performers, with PAX6 treatment resulting in slightly more c-peptide than SIX2. However, it should be noted that the mean brightness observed for each image—18.212 and 17.579 for PAX6 and SIX2 respectively—did not display a large difference in comparison to the difference observed between both of these transcription factors and the wild type cells. PAX6, while notable for its role in the development of neural tissue, is also important in the function of fully mature pancreatic beta cells [38]. SIX2, similarly, is involved in limb development, but also appears to be linked to insulin production and glucose sensing [39]. By pairing these two

transcription factors in a future study, even more efficient differentiation may be possible, but further investigation is needed to draw more solid conclusions regarding this topic.

Possible Limitations of a modRNA Approach

Many potential benefits of a modRNA approach to pancreatic beta cell differentiation were outlined earlier in this study, but it is also important to note that there are some potential difficulties to implementing this method on a large scale. For the method to be feasible, it must be at least as efficient as existing methods of pancreatic beta cell differentiation, but as seen in Figure 3, production of c-peptide was a scattered phenomenon at best. Even the highest-performing transcription factors were not able to facilitate the differentiation of a large amount of cells, and while this study only includes an examination of *individual* transcription factors, it will still likely take a good deal of time and effort for a sufficiently efficient combination to be found. It is even possible that *no* combination of the eleven transcription factors discussed here will be meaningfully efficient, which would necessitate the inclusion of even more transcription factors.

Additionally, modRNA is known to be a bit unstable both in and out of biological systems, which can make it difficult to work with in the lab [40]. Production of modRNA needs to be done in a controlled environment where contact with RNases is minimized and the possibility of contamination with other RNA is low. This often requires the dedication of a particular hood in the lab solely to the purpose of working with RNA, which needs to be meticulously maintained. The process of producing modRNA can also be a bit labor-intensive, especially if several different transcription factors are involved. Once the initial batch is made,

the difficulty decreases somewhat, but it will still require the work of at least one person, since it is unlikely that the process could be automated at this time.

Topics for Further Study

As mentioned earlier in this section, many more combinations of transcription factors need to be tested to determine which, if any, result in the most efficient differentiation. In addition to the combinations described above, it may also be prudent to do a test of all eleven transcription factors at once, as well as any other combinations that are suggested either by the results of more experiments or a search of the existing literature. If a successful combination is found, a direct comparison with the current beta cell differentiation protocols would also be helpful to assess whether the modRNA approach is truly more efficient than what is currently being used in labs.

Altering the method of transfection could be another interesting topic of study. Lipofection appeared to work for the purposes of this experiment, but it is possible that other methods would result in more efficient transfection, which could deliver more modRNA into cells and increase the likelihood of cell differentiation.

Chapter 6

Conclusion

In this study, lipofection was found to be a potentially effective way of delivering modRNA into iPSCs, even if many of the cells perished in the 24-hour period between transfection and imaging. When comparing the bright field images of three difference cell sites of PDX1-transfected cells with the GFP image, a clear overlap between the location of the cells and the expression of fluorescence was observed. It's possible that other methods of transfection would be equally effective, but lipofection produces results without many of the negative effects of transfection methods such as the viral vector method.

None of the eleven transcription factors included in this study were independently sufficient to facilitate widespread differentiation of iPSCs into c-peptide producing pancreatic beta cells, but many of them resulted in the production of at least *some* cells expressing c-peptide. PAX6 and SIX2, in particular, showed noticeable expression of c-peptide, and every transcription factor studied had a relative brightness greater than one, indicating that they produced more c-peptide than the wild type cells. While no transcription factor alone is enough for a feasible modRNA differentiation protocol, many of them hold promise, and further study is required to determine what combinations, if any, will prove effective.

In conclusion, modRNA holds great promise as a facilitator of differentiation of iPSCs into pancreatic beta cells, but it will take much more work for that potential to be realized.

BIBLIOGRAPHY

1. Daneman, D. (2006). Type 1 diabetes. *The Lancet*, *367*(9513), 847–858. https://doi.org/10.1016/s0140-6736(06)68341-4
2. Lawrence, J. M., Divers, J., Isom, S., Saydah, S., Imperatore, G., Pihoker, C., Marcovina, S. M., Mayer-Davis, E. J., Hamman, R. F., Dolan, L., Dabelea, D., Pettitt, D. J., & Liese, A. D. (2021). Trends in prevalence of type 1 and type 2 diabetes in children and adolescents in the US, 2001-2017. *JAMA*, *326*(8), 717. https://doi.org/10.1001/jama.2021.11165
3. Onkamo, P., Väänänen, S., Karvonen, M., & Tuomilehto, J. (1999). Worldwide increase in incidence of type I diabetes - the analysis of the data on published incidence trends. *Diabetologia*, *42*(12), 1395–1403. https://doi.org/10.1007/s001250051309
4. Egro, F. M. (2013). Why is type 1 diabetes increasing? *Journal of Molecular Endocrinology*, *51*(1). https://doi.org/10.1530/jme-13-0067
5. Leete, P., Willcox, A., Krogvold, L., Dahl-Jørgensen, K., Foulis, A. K., Richardson, S. J., & Morgan, N. G. (2016). Differential insulitic profiles determine the extent of β-cell destruction and the age at onset of type 1 diabetes. *Diabetes*, *65*(5), 1362–1369. https://doi.org/10.2337/db15-1615
6. Atkinson, M. A., Eisenbarth, G. S., & Michels, A. W. (2014). Type 1 diabetes. *The Lancet*, *383*(9911), 69–82. https://doi.org/10.1016/s0140-6736(13)60591-7

7. Gale, E. A. M. (2004). European Nicotinamide Diabetes Intervention Trial (Endit): A randomised controlled trial of intervention before the onset of type 1 diabetes. *The Lancet*, *363*(9413), 925–931. https://doi.org/10.1016/s0140-6736(04)15786-3
8. Perkins, B. A., Bebu, I., de Boer, I. H., Molitch, M., Tamborlane, W., Lorenzi, G., Herman, W., White, N. H., Pop-Busui, R., Paterson, A. D., Orchard, T., Cowie, C., & Lachin, J. M. (2019). Risk factors for kidney disease in type 1 diabetes. *Diabetes Care*, *42*(5), 883–890. https://doi.org/10.2337/dc18-2062
9. Groop, P.-H., Thomas, M. C., Moran, J. L., Wadèn, J., Thorn, L. M., Mäkinen, V.-P., Rosengård-Bärlund, M., Saraheimo, M., Hietala, K., Heikkilä, O., & Forsblom, C. (2009). The presence and severity of chronic kidney disease predicts all-cause mortality in type 1 diabetes. *Diabetes*, *58*(7), 1651–1658. https://doi.org/10.2337/db08-1543
10. Corbin, K. D., Driscoll, K. A., Pratley, R. E., Smith, S. R., Maahs, D. M., & Mayer-Davis, E. J. (2018). Obesity in type 1 diabetes: Pathophysiology, clinical impact, and Mechanisms. *Endocrine Reviews*, *39*(5), 629–663. https://doi.org/10.1210/er.2017-00191
11. Osuchowski, M. F., Craciun, F. L., Schuller, E., Sima, C., Gyurko, R., & Remick, D. G. (2010). Untreated type 1 diabetes increases sepsis-induced mortality without inducing a prelethal cytokine response. *Shock*, *34*(4), 369–376. https://doi.org/10.1097/shk.0b013e3181dc40a8
12. Gan, M. J., Albanese-O'Neill, A., & Haller, M. J. (2012). Type 1 diabetes: Current concepts in epidemiology, pathophysiology, clinical care, and research. *Current Problems in Pediatric and Adolescent Health Care*, *42*(10), 269–291. https://doi.org/10.1016/j.cppeds.2012.07.002

13. Brown, S. A., Kovatchev, B. P., Raghinaru, D., Lum, J. W., Buckingham, B. A., Kudva, Y. C., Laffel, L. M., Levy, C. J., Pinsker, J. E., Wadwa, R. P., Dassau, E., Doyle, F. J., Anderson, S. M., Church, M. M., Dadlani, V., Ekhlaspour, L., Forlenza, G. P., Isganaitis, E., Lam, D. W., … Beck, R. W. (2019). Six-month randomized, Multicenter Trial of closed-loop control in type 1 diabetes. *New England Journal of Medicine*, *381*(18), 1707–1717. https://doi.org/10.1056/nejmoa1907863

14. Ly, T. T., Roy, A., Grosman, B., Shin, J., Campbell, A., Monirabbasi, S., Liang, B., von Eyben, R., Shanmugham, S., Clinton, P., & Buckingham, B. A. (2015). Day and night closed-loop control using the integrated Medtronic Hybrid closed-loop system in type 1 diabetes at diabetes camp. *Diabetes Care*, *38*(7), 1205–1211. https://doi.org/10.2337/dc14-3073

15. Giani, E., Snelgrove, R., Volkening, L. K., & Laffel, L. M. (2016). Continuous glucose monitoring (CGM) adherence in youth with type 1 diabetes: Associations with biomedical and psychosocial variables. *Journal of Diabetes Science and Technology*, *11*(3), 476–483. https://doi.org/10.1177/1932296816676280

16. Maehr, R., Chen, S., Snitow, M., Ludwig, T., Yagasaki, L., Goland, R., Leibel, R. L., & Melton, D. A. (2009). Generation of pluripotent stem cells from patients with type 1 diabetes. *Proceedings of the National Academy of Sciences*, *106*(37), 15768–15773. https://doi.org/10.1073/pnas.0906894106

17. Sui, L., Danzl, N., Campbell, S. R., Viola, R., Williams, D., Xing, Y., Wang, Y., Phillips, N., Poffenberger, G., Johannesson, B., Oberholzer, J., Powers, A. C., Leibel, R. L., Chen, X., Sykes, M., & Egli, D. (2017). B-cell replacement in mice using human type 1

diabetes nuclear transfer embryonic stem cells. *Diabetes*, *67*(1), 26–35. https://doi.org/10.2337/db17-0120

18. Zickri, M. B., Aboul-Fotouh, G. I., Omar, A. I., El-Shafei, A. A., & Reda, A. M. (2018). Effect of stem cells and gene transfected stem cells therapy on the pancreas of experimentally induced type 1 diabetes. *International Journal of Stem Cells*, *11*(2), 205–215. https://doi.org/10.15283/ijsc18002
19. Maxwell, K. G., & Millman, J. R. (2021). Applications of ipsc-derived beta cells from patients with diabetes. *Cell Reports Medicine*, *2*(4), 100238. https://doi.org/10.1016/j.xcrm.2021.100238
20. Yoshihara, E., O'Connor, C., Gasser, E., Wei, Z., Oh, T. G., Tseng, T. W., Wang, D., Cayabyab, F., Dai, Y., Yu, R. T., Liddle, C., Atkins, A. R., Downes, M., & Evans, R. M. (2020).
21. Vethe, H., Ghila, L., Berle, M., Hoareau, L., Haaland, Ø. A., Scholz, H., Paulo, J. A., Chera, S., & Ræder, H. (2019). The effect of Wnt pathway modulators on human ipsc-derived pancreatic beta cell maturation. *Frontiers in Endocrinology*, *10*. https://doi.org/10.3389/fendo.2019.00293
22. Warren, L., Manos, P. D., Ahfeldt, T., Loh, Y.-H., Li, H., Lau, F., Ebina, W., Mandal, P. K., Smith, Z. D., Meissner, A., Daley, G. Q., Brack, A. S., Collins, J. J., Cowan, C., Schlaeger, T. M., & Rossi, D. J. (2010). Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mrna. *Cell Stem Cell*, *7*(5), 618–630. https://doi.org/10.1016/j.stem.2010.08.012
23. Hiratsuka, K., Monkawa, T., Akiyama, T., Nakatake, Y., Oda, M., Goparaju, S. K., Kimura, H., Chikazawa-Nohtomi, N., Sato, S., Ishiguro, K., Yamaguchi, S., Suzuki, S.,

Morizane, R., Ko, S. B., Itoh, H., & Ko, M. S. (2019). Induction of human pluripotent stem cells into kidney tissues by synthetic mrnas encoding transcription factors. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-018-37485-8

24. Wang, K., Lin, R.-Z., Hong, X., Ng, A. H., Lee, C. N., Neumeyer, J., Wang, G., Wang, X., Ma, M., Pu, W. T., Church, G. M., & Melero-Martin, J. M. (2020). Robust differentiation of human pluripotent stem cells into endothelial cells via temporal modulation of ETV2 with modified mrna. *Science Advances*, *6*(30). https://doi.org/10.1126/sciadv.aba7606
25. Gutierrez-Aranda, I., Ramos-Mejia, V., Bueno, C., Munoz-Lopez, M., Real, P. J., Mácia, A., Sanchez, L., Ligero, G., Garcia-Parez, J. L., & Menendez, P. (2010). Human induced pluripotent stem cells develop teratoma more efficiently and faster than human embryonic stem cells regardless the site of injection. *Stem Cells*, *28*(9), 1568–1570. https://doi.org/10.1002/stem.471
26. U.S. National Library of Medicine. (n.d.). *Home - gene - NCBI*. National Center for Biotechnology Information. Retrieved March 14, 2022, from https://www.ncbi.nlm.nih.gov/gene/
27. Alessandro Howells. (2022) GFP and c-peptide images.
28. Evers, M. J. W., Du, W., Yang, Q., Kooijmans, S. A. A., Vink, A., van Steenbergen, M., Vader, P., de Jager, S. C. A., Fuchs, S. A., Mastrobattista, E., Sluijter, J. P. G., Lei, Z., & Schiffelers, R. (2022). Delivery of modified mrna to damaged myocardium by systemic administration of lipid nanoparticles. *Journal of Controlled Release*, *343*, 207–216. https://doi.org/10.1016/j.jconrel.2022.01.027

29. Ribeiro, S. C., Mendes, R., Madeira, C., Monteiro, G. A., da Silva, C. L., & Cabral, J. M. S. (2010). A quantitative method to evaluate mesenchymal stem cell lipofection using real-time PCR. *Biotechnology Progress*, *26*(5), 1501–1504. https://doi.org/10.1002/btpr.451

30. Hadas, Y., Sultana, N., Youssef, E., Sharkar, M. T., Kaur, K., Chepurko, E., & Zangi, L. (2019). Optimizing modified mrna in vitro synthesis protocol for heart gene therapy. *Molecular Therapy - Methods & Clinical Development*, *14*, 300–305. https://doi.org/10.1016/j.omtm.2019.07.006

31. U.S. National Library of Medicine. (2020, December 3). *C-peptide test: Medlineplus medical test*. MedlinePlus. Retrieved March 14, 2022, from https://medlineplus.gov/lab-tests/c-peptide-test/

32. Gao, N., LeLay, J., Vatamaniuk, M. Z., Rieck, S., Friedman, J. R., & Kaestner, K. H. (2008). Dynamic regulation of PDX1 enhancers by Foxa1 and FOXA2 is essential for pancreas development. *Genes & Development*, *22*(24), 3435–3448. https://doi.org/10.1101/gad.1752608

33. Zhu, Y., Liu, Q., Zhou, Z., & Ikeda, Y. (2017). PDX1, neurogenin-3, and MAFA: Critical transcription regulators for beta cell development and regeneration. *Stem Cell Research & Therapy*, *8*(1). https://doi.org/10.1186/s13287-017-0694-z

34. Guo, T., Wang, W., Zhang, H., Liu, Y., Chen, P., Ma, K., & Zhou, C. (2011). ISL1 promotes pancreatic islet cell proliferation. *PLoS ONE*, *6*(8). https://doi.org/10.1371/journal.pone.0022387

35. Papizan, J. B., Singer, R. A., Tschen, S.-I., Dhawan, S., Friel, J. M., Hipkens, S. B., Magnuson, M. A., Bhushan, A., & Sussel, L. (2011). Nkx2.2 repressor complex regulates

islet β-cell specification and prevents β-to-α-cell reprogramming. *Genes & Development*, *25*(21), 2291–2305. https://doi.org/10.1101/gad.173039.111

36. Nelson, S. B., Schaffer, A. E., & Sander, M. (2007). The transcription factors NKX6.1 and NKX6.2 possess equivalent activities in promoting beta-cell fate specification in PDX1+ pancreatic progenitor cells. *Development*, *134*(13), 2491–2500. https://doi.org/10.1242/dev.002691
37. Romer, A. I., Singer, R. A., Sui, L., Egli, D., & Sussel, L. (2019). Murine perinatal β-cell proliferation and the differentiation of human stem cell–derived insulin-expressing cells require neurod1. *Diabetes*, *68*(12), 2259–2271. https://doi.org/10.2337/db19-0117
38. Ashery-Padan, R., Zhou, X., Marquardt, T., Herrera, P., Toube, L., Berry, A., & Gruss, P. (2004). Conditional inactivation of PAX6 in the pancreas causes early onset of diabetes. *Developmental Biology*, *269*(2), 479–488. https://doi.org/10.1016/j.ydbio.2004.01.040
39. Bevacqua, R. J., Lam, J. Y., Peiris, H., Whitener, R. L., Kim, S., Gu, X., Friedlander, M. S. H., & Kim, S. K. (2021). SIX2 and SIX3 coordinately regulate functional maturity and fate of human pancreatic β cells. *Genes & Development*, *35*(3-4), 234–249. https://doi.org/10.1101/gad.342378.120
40. Liew, L. C., Ho, B. X., & Soh, B.-S. (2020). Mending a broken heart: Current strategies and limitations of cell-based therapy. Stem Cell Research & Therapy, 11(1). https://doi.org/10.1186/s13287-020-01648-0

ACADEMIC VITA

Education

Senior in the Schreyer Honors College at The Pennsylvania State University, University Park, PA

Bachelor of Science in Biomedical Engineering

Expected Graduation: May 2022

Lab Experience

- *The Thomas Lab* Spring 2020
  - Maintained fly cultures
  - Captured images of developing *Drosophila melanogaster* embryos under a microscope
- *The Lian Lab* Fall 2020—Present
  - Generated modRNA for experimental use
  - Cultured *E. coli* to produce plasmids
  - Prepared samples for DNA sequencing
  - Performed ligation and transformation in *E. coli* plasmids
  - Used gel electrophoresis to identify DNA fragments

Publications

- Robust genome and RNA editing via CRISPR nucleases in PiggyBac systems. (2022) Authors: Yuqian Jiang, Rachel Hoenisch, Yun Chang, Xiaoping Bao, Craig Cameron, Xiaojun Lance Lian.

Honors and Activities

- *Penn State International Affairs and Debate Association (PSIADA)*
  - Head of Committee for the Pennsylvania High School United Nations Conference (2020)
  - Technology Chair on the Executive Board (2020-2021)
    - PUNC XIII Director of Technology
    - PHUNC IX Director of Technology
  - Integrity Chair on the Executive Board (2021-2022)
- *Penn State Dean's List*, 2018-2021

Skills

PCR, Gel Electrophoresis, MATLAB, COMSOL, Python