Published in final edited form as:

Regen Eng Transl Med. 2019 March ; 5(1): 42–52. doi:10.1007/s40883-018-0082-y.

Human beta cells generated from pluripotent stem cells or cellular reprogramming for curing diabetes

Lauren N. Randolph^{1,2}, Agamoni Bhattacharyya^{1,2}, and Xiaojun Lance Lian^{1,2,3,*}

¹Department of Biomedical Engineering, Pennsylvania State University, University Park, PA, 16802, USA

²The Huck Institutes of the Life Sciences, Pennsylvania State University, University Park, PA, 16802, USA

³Department of Biology, Pennsylvania State University, University Park, PA, 16802, USA

Abstract

Diabetes is a group of metabolic diseases characterized by aberrantly high blood glucose levels caused by defects in insulin secretion, its action, or both, which affects approximately 30.3 million people (9.4% of the population) in the United States. This review will focus on using human β cells to treat and cure diabetes because β cells are absent, due to an autoimmune destruction, in Type 1 diabetes or dysfunctional in Type 2 diabetes. In order to generate enough functional β cells for diabetes treatment (0.1 to 1 billion cells to treat one patient), a basic science approach by mimicking what happens in normal pancreatic development must be closely aligned with engineering. Two general approaches are discussed here. The first one uses human pluripotent stem cells (hPSCs) to perform directed differentiation of hPSCs to β cells. This is advantageous because hPSCs grow indefinitely, providing a virtually unlimited source of material. Therefore, if we develop an efficient β cell differentiation protocol, we can essentially generate an unlimited amount of β cells for disease modeling and diabetes treatment. The second approach is cellular reprogramming, with which we may begin with any cell type and convert it directly into a β cell. The success of this cellular reprogramming approach, however, depends on the discovery of a robust and efficient transcription factor cocktail that can ignite this process, similar to what has been achieved in generating induced pluripotent stem cells. This discovery should be possible through identifying the important transcription factors and pioneer factors via recent advances in single-cell RNA sequencing. In short, a new renaissance in pancreas developmental biology, stem cell engineering, and cellular reprogramming for curing diabetes appears to be on the horizon.

Introduction

Regenerative medicine and tissue engineering are decades old concepts that are contrastingly still in their infancy when it comes to scientific achievement and clinical application. Clinical standards for replacing lost tissue remain focused on autologous and allogeneic transplant, for which the high risk associated with the lack of donor tissue, chance of developing graft versus host disease, and complications arising from immunosuppressive

*Correspondence should be addressed to: Xiaojun Lance Lian (Lian@psu.edu).

regimens leaves much to be desired. The introduction of human embryonic stem cell (hESC) derivation technology and culture techniques¹, combined with the identification of reprogramming strategies to generate induced pluripotent stem cells (iPSCs)^{2–4}, has revolutionized the field and sparked efforts to de novo generate high demand tissues, such as neurons, cardiomyocytes, or cells. Some cell types, such as cardiomyocytes have been successfully generated with defined directed differentiation protocols and await further refinement to translate this technology to clinical application^{5–7}. Other cell types, such as pancreatic β cells, have remained more challenging. These cells have high impact potential for clinical translation and treatment of type I diabetes; however the developmental stages of β cell specification are comparatively complex.

There are two general strategies under investigation for generating human pancreatic β cells. One method is directed differentiation of human pluripotent stem cells (hPSCs), including hESCs and iPSCs, which uses growth factors and small molecules to influence the path of differentiation of hPSCs by mimicking developmental stages and stimuli. The other method is cellular reprogramming of other somatic cells to a β cell fate by promoting the expression of a defined cocktail of pioneer factors and transcription factors to stimulate transdifferentiation. Both strategies require in depth understanding of pancreatic β cell development in human embryogenesis as well as identification of molecular biomarkers of different progenitor states. Furthermore, definitive cell state and functional assays are required to ascertain that the cells derived are indeed the desired cell type.

What follows comprises a detailed review of the natural development, directed differentiation strategies, and reprogramming efforts aimed at generating β cells to date as well as future directions and challenges facing the field.

What are β cells and Type 1 diabetes?

The pancreas is a vital organ in the human body that is responsible for secreting hormones necessary to regulate blood glucose levels, as well as producing enzymes for digestion. The pancreas is made up of highly specialized cells that perform either endocrine or exocrine functions. Exocrine cells, including acinar and duct cells, produce enzymes for digestion of food⁸. The endocrine cells are responsible for secreting different hormones and are localized to the islets of Langerhans, which are clusters of unique cells each of which secrete a different hormone. Glucagon is secreted by α cells and is responsible for increasing the concentration of glucose in the blood when necessary⁹. Somatostatin is secreted by δ cells¹⁰. Pancreatic polypeptide is secreted by γ cells or PP cells and is responsible for regulating food intake, and ghrelin is secreted by ϵ cells in response to hunger or an empty stomach^{11,12}. Insulin is secreted by β cells and is a prominent pancreatic hormone necessary to regulate glucose homeostasis (Fig. 1). It is responsible for transporting glucose from the bloodstream into cells, where it is converted into cellular fuel (ATP). As is the case with many human proteins, simple transcription and translation of insulin is not sufficient to obtain functional insulin. Post-translational processing and responsive secretion are critical components to the production of insulin. Transcription of the insulin gene results in translation of a protein called preproinsulin. Once the N-terminus has been removed, preproinsulin becomes proinsulin¹³. The c-peptide is then cut off from proinsulin to generate

insulin, which is stored in the secretory granules of β cells¹³. As c-peptide is a byproduct of insulin production indicating successful post-translational modification, it is a useful marker for determining how much insulin is produced inside a given cell.

Type 1 diabetes results from insulin deficiency caused by the loss of β cells as the result of an aberrant autoimmune response¹⁴. Initially, β cell auto-antigens are recognized by macrophages, dendritic cells, and B lymphocytes¹⁴. These auto-antigens are then processed by T lymphocytes, which secrete cytokines to activate β -cell specific T lymphocytes¹⁴. These T cells are then recruited to pancreatic islets and produce more cytokines, activating macrophages and additional T cells, eventually destroying the β cells¹⁴. Due to the destruction of the β cell population, the secretion of insulin dramatically decreases, preventing glucose from leaving the bloodstream to enter cells, resulting in hyperglycemic blood glucose levels.

Clinical need for developing better therapies for diabetes

Type I diabetes is diagnosed in children and young adults and accounts for approximately 5% of people with diabetes¹⁴. The prevalence of type 1 diabetes is increasing worldwide at a rate of approximately 3%¹⁵. If untreated, patients with type 1 diabetes can die prematurely or have prolonged ill health due to severe complications¹⁶. One in three patients with type 1 diabetes have hypertension, which can significantly increase the occurrence of a stroke or cardiovascular disease¹⁷. Therefore, a type 1 diabetic patient will most likely develop multiple health problems, creating a compounding financial burden on the health care system and increasing the need for treatment and a cure.

Currently, methods to treat type 1 diabetes include injection of insulin or pancreatic islet transplants. However, injecting insulin does not cure the disease and it requires patients to monitor their blood glucose levels at all time. It has been shown to cost 30,000 patients about \$10 billion over their lifetime¹⁸. This means that an average type 1 diabetic patient will spend over \$300,000 on insulin, insulin pumps, and other equipment required to maintain regular glucose levels. Alternatively, another current treatment method relies on using cadaver islet cells for transplantation. While the cadaver islet cell transplant has shown success, there is a shortage of human pancreatic islets available, as is the case with any human cell or tissue transplant, and it requires lifetime immunosuppressive therapy to allow transplanted islet cell survival. Long-term immunosuppression can cause a variety of complications and may not be successful in ensuring transplant survival, which could necessitate a second transplant. As a result, many researchers and clinicians are looking towards tissue engineering and regenerative medicine strategies to identify a viable alternative source of functional β cells. With the expansion of stem cell technology and the increased evidence of successful cellular reprogramming, the ability to de-novo generate functional β cells appears to be closer on the horizon.

β Cell Development

Pancreatic development takes a relatively long time during embryogenesis compared to many other organs. The fully functional pancreas does not appear until postnatal day 8 in

mice and greater than gestational week 20 in humans¹⁹. Compared to the heart which is fully formed after 10 gestational weeks in a human, the pancreas, and thus functional β cells, emerge late in development²⁰. This can be indicative of complex developmental signaling cues directing the differentiation process.

The cells of the pancreas are derived from the endoderm germ layer, and as such, the first step of pancreatic development is definitive endoderm specification. This event is often triggered by increased TGF β superfamily signaling, in particular through Nodal or Activin A treatment²¹. The generation of the definitive endoderm is marked by the emergence of SOX17 and FOXA2 expression. Other tissues derived from the definitive endoderm include the liver, intestinal, and stomach tissues^{19,21}, making them strong candidates for reprogramming strategies due to their common early progenitor state.

Following the emergence of the definitive endoderm, the pancreatic endoderm is marked by the expression of PDX1, NKX6.1, SOX9, and PTF1A²¹. The pancreatic endoderm is a further differentiated subset of the definitive endoderm comprising all progenitor cells of the pancreas. The emergence of PDX1 expression is crucial for this transition and lineage tracing studies have shown that it is a master regulator of the pancreatic cell fate as all pancreatic cells are derived from PDX1 positive progenitors²². The emergence of the pancreatic endoderm requires passing through a primitive gut tube and posterior foregut stage by activating retinoic acid (RA) signaling and FGF signaling, but inhibiting sonic hedgehog signaling, which is provided *in vivo* by proximity to the developing notochord^{23,24}.

At this point the pancreatic endoderm cells will further split to form either the exocrine or endocrine lineages. The exocrine duct cells will retain SOX9 expression and acinar cells will remain PTF1A positive¹⁹. These cells will localize at the tips of the bud-like structures of the developing pancreas, whereas emergent endocrine cells will form the trunks of these buds¹⁹. Endocrine progenitor cells will maintain expression of PDX1 and NKX6.1, but will also show emergent expression of NGN3 and NEUROD1¹⁹. As the pancreas develops from definitive endoderm that is connected to the notochord, it is known to have some shared molecular characteristics with neurons²⁵. In addition, pancreatic endocrine cells develop in a scatter fashion within a field of progenitor cells, similar to neurons in the central nervous system²⁵. Due to the previously mentioned proximity to the notochord and some shared molecular characteristics with neurons, such as NEUROD1 expression, Apelqvist et al. tested whether Notch signaling is involved in pancreatic development in mice. They found that *Ngn3* is a pro-endocrine gene and is expressed during reduced Notch signaling²⁵. In addition, Notch signaling is essential to differentiate between endocrine and exocrine cells in developing pancreas; more importantly, Notch signaling shows a bimodal role in this specification process with activation in exocrine development and inhibition in endocrine cells²⁵.

From this endocrine progenitor state, the cells will become further differentiated by the expression of a cell specific hormone, acquire unique cellular appearance, and organize into islet clusters. In addition to showing transcriptomic hallmarks of pancreatic endocrine cells,

β cells are characterized by their functional secretion of insulin in response to increased blood glucose levels.

Directed differentiation of human pluripotent stem cells to β -like cells

One of the first attempts to direct differentiation of mouse embryonic stem cells (mESCs) to β cells was done in 2001 by Lumelsky et al.²⁶. Their strategy was formed by the observation that the pancreas develops from definitive endoderm, which is connected to the notochord, and by previous experiments differentiating mESCs into neural cells. They predicted that Nestin-positive cells, a marker usually found in neural precursors, would be easily directed to a pancreatic cell fate²⁶. In their procedure, they isolated Nestin-positive cells using serum deprivation and then expanded the cells with basic fibroblast growth factor (bFGF) in N2 serum free medium with B-27 supplement²⁶. After six days, they removed the bFGF to promote the end of cell division and differentiation and added nicotinamide, which is known to increase the production of insulin and is an inducer of endocrine differentiation in human fetal pancreatic cells^{26,27}. Furthermore, recent work has highlighted the importance of nicotinamide in the generation of β -like cells *in vitro* showing a concentration dependent effect on the differentiation efficiency²⁸. Lumelsky et al. were able to generate pancreatic cells secreting about fifty times less insulin per cell than normal β cells²⁶. In addition, after grafting the insulin-producing cells into the shoulder of streptozotocin induced diabetic mice, they found that there was no long-term correction for hyperglycemia²⁶. As one of the first directed differentiation strategies developed to generate insulin-producing β -like cells, this work illustrates the importance of the developmental microenvironment as well as the use of nicotinamide to induce pancreatic endocrine differentiation. However, this procedure does not adhere to the developmental pathway of the human pancreas, as described earlier, a potential cause of incomplete functional differentiation.

Between the years 2005–2008, the Baetge group developed techniques to differentiate hESCs into pancreatic insulin-secreting endocrine cells by mimicking embryonic development through the manipulation of key signaling pathways: FGF, SHH, TGF β , WNT, Notch, and RA. In 2005, they first developed an efficient method to differentiate hESCs into definitive endoderm cells. Using low serum media supplemented with Activin A in a monolayer culture with feeder cells for 5 days, they were able to differentiate seven different hESC lines to the definitive endoderm, with varying efficiencies (up to 80%) as measured by SOX17 expression²⁹. They did show that CXCR4 expression based sorting could enrich the cells to a relatively pure definitive endoderm population²⁹. However, the variance in efficiencies across cell lines due to differences in the inclination of the primitive streak/mesendoderm to convert to definitive endoderm or other yet un-investigated factors remains a challenge in efficient definitive endoderm differentiation.

In 2006, the Baetge group expanded on their definitive endoderm protocol to produce endocrine cells that secrete insulin in response to elevated glucose levels³⁰. The definitive endoderm cells are differentiated into the primitive gut tube and then into the posterior foregut by RA addition, activating the retinoic acid signaling pathway, as well as the addition of sonic hedgehog inhibitor cyclopamine, inhibiting the hedgehog pathway, and FGF10 addition, which promotes progenitor population expansion³⁰. They noted that the

absence of RA in their differentiation still lead to the expression of PDX1; however other key pancreatic and β cell specific markers such as NGN3 and insulin were not upregulated, highlighting the key role of RA signaling for β cell differentiation³⁰. Likewise, the omission of cyclopamine led to lower transcription of insulin genes indicating an important role for the spatial organization of the developing embryo³⁰. Their *in vitro* differentiated cells could process 64% of the proinsulin content, meaning that post-translational processing machinery was not present³⁰. In addition, the differentiated cells were not mature because they could not switch from MAFB expression to MAFA expression, making them more similar to immature fetal β cells³⁰.

In 2008, the Baetge group improved the strategy developed by D'Amour et al. and tested it *in vivo* with immune-compromised mice³¹. This strategy incorporated Noggin, a BMP pathway inhibitor, into the posterior foregut stage of differentiation, indicating a biphasic role for TGF- β signaling that was previously unexplored in this context³¹. In order to produce hormone secreting pancreatic endocrine cells, they used an *in vivo* maturation step by transplanting *in vitro* derived pancreatic endoderm cells into immune-compromised mice³¹. Approximately three months after implantation, roughly 92% of the implanted mice achieved maximal insulin secretion at human levels, which was sufficient to protect against streptozotocin-induced hyperglycemia³¹. The novelty of this method lies in the use of Noggin and an *in vivo* maturation step. The transplantation of pancreatic progenitor cells may lead to teratoma formation resulting from contaminating undifferentiated cells. While, this protocol further clarified intermediate steps of pancreatic β cell differentiation, it leaves much to be desired in terms of β cell specification and clinical relevance.

In 2007, Jiang et al. developed a serum-free protocol to generate insulin-producing islet-like clusters from hESCs grown in feeder-free conditions. In their protocol, they convert hESCs to definitive endoderm using Activin A and sodium butyrate, which makes cells more responsive to Activin A³². To avoid using serum, they tested different amounts/concentrations of sodium butyrate with Activin A and were successfully able to convert 64.9% of the cells to SOX17⁺ cells³². After further differentiation, they found that 15–25% of the cells expressed PDX1, meaning less than a quarter of the definitive endoderm cells were successfully differentiated to pancreatic progenitor³². Upon generating hormone producing cells, the cells showed positive expression of c-peptide, glucagon, and somatostatin, indicating the generation of poly-hormonal cells. Their differentiated populations were also contaminated with pancreatic ductal cells, meaning that their protocol was not specifically producing endocrine cells. This protocol moved towards clinically relevant differentiation techniques in the use of feeder-free and serum-free conditions; however, their protocol showed very low yields of c-peptide⁺ polyhormonal cells as well as pancreatic exocrine cells, indicating issues with the endocrine specification stages of their differentiation.

Due to low insulin-secretion in differentiated cells from previous experiments, optimizing the activation and inhibition of discrete signaling pathways has been the subject of intense investigation. In 2011, Dr. Gordon Keller's group analyzed the influence of BMP, activin/nodal, and WNT signaling on the outcomes from the D'Amour/Kroon protocol^{30,31,33}. They manipulated the canonical WNT pathway, using Wnt3a ligands for activation and DKK1 for

inhibition, during the differentiation stages mimicking gut tube formation and posterior foregut specification. They found that Wnt inhibition at this stage was detrimental to endocrine development; however there is an optimal concentration range for Wnt3a, which increases downstream insulin production³³. When optimizing the pancreatic specification stage of differentiation, Keller et al. found it was essential to inhibit the BMP and TGF β /activin/nodal pathways to ensure endocrine commitment. Therefore, they used Noggin and SB431542, which is another TGF β inhibitor, in their strategy³⁴. They were able to successfully produce 25% c-peptide⁺ cells with insulin secretion levels similar to that of the adult pancreas after temporal modulation of the WNT, BMP, and TGF β /activin/nodal pathways at different points of the development³³. This represented a significant leap in understanding early specification of pancreatic endocrine cells and further unveiled the complexity of the signaling pathway networks at play.

In 2015, Dr. Gordon Keller's group further improved their previous protocol to promote the production of NKX6.1⁺ progenitor cells³⁵ (Fig. 2). They noted that, unlike in murine development, polyhormonal and monohormonal pancreatic endocrine cells arise from distinct developmental programs in humans, a plausible reason why their previous protocol was suboptimal³⁶. As such, they modified the endocrine cell specification stage of their previous method by culturing the cells for various days with EGF, Noggin, and nicotinamide, resulting in a population that was 70% NKX6.1⁺. The addition of nicotinamide has previously been shown to increase the population of insulin secreting cells²⁷. The Kroon/D'Amour protocol does not use EGF and nicotinamide; however, the development of NKX6.1⁺ progenitors without the use of these two factors is cell line dependent^{30,31}. In contrast, when EGF and nicotinamide are used, the production of NKX6.1⁺ progenitor cells is not cell line dependent³⁵. Furthermore, they noted that the inhibition or activation of the TGF β pathway is dependent on the cell line being differentiated³⁵. A high degree of cell line variability is one of the key challenges in the field, which could lead to difficulty when translating directed differentiation technology to clinical use.

In 2014, Rezanian et al. optimized their previous protocols to enhance the production of mature pancreatic beta cells, distinguished by MAFA⁺ cells³⁷. Their seven-stage protocol optimized the generation of PDX1⁺/NKX6.1⁺ cells by introducing Vitamin C during development of the primitive gut tube to the pancreatic endoderm³⁷. Vitamin C also reduced the expression of NGN3, which they noted was vital in early stages of differentiation to produce functional β cells that are not polyhormonal³⁷. They optimized the production of NKX6.1⁺/NEUROD1⁺ cells by using an ALK5 inhibitor (ALK5iII) and T3, thyroid hormone³⁷. After the addition of gamma secretase inhibitor XX (GSI XX), a Notch pathway inhibitor, they increased the percentage of NKX6.1⁺/insulin⁺/glucagon⁻ cells (immature beta cells) from 25% to 50%³⁷. To enhance the immature beta cells to mature beta cells, they identified R428, a small molecule inhibitor of the tyrosine kinase receptor AXL, as an inducer of MAFA⁺ mature beta cells³⁷. Within 6 weeks after transplantation of these mature beta cells into diabetic immunodeficient mice, the c-peptide levels achieved were comparable to those in diabetic mice transplanted with human islets³⁷. However, they noticed a delay in glucose response, implying that the cells are functionally immature, even though the cells are expressing MAFA. This method offers high efficiency in differentiated

mature beta cells, although further developments are required for rapid insulin secretory responses³⁷.

In 2014, Dr. Douglas Melton's group developed a method to produce functional mature β cells from hESC line HUES8 and human iPSC lines (hiPSC-1 and hiPSC-2)³⁸ (Fig. 2). To increase the population of pancreatic progenitor cells, they introduced the use of SANT1, which is a sonic hedgehog signaling pathway inhibitor³⁸. After the production of pancreatic progenitor cells, they added numerous factors, such as T3 (thyroid hormone), XXI (gamma-secretase inhibitor), heparin, Alk5 receptor inhibitor II, and betacellulin (EGF family member), to promote endocrine and then β cell specification (Fig. 2). Their protocol produced islet-like clusters that respond to high glucose treatment with a frequency of 75% *in vitro*, while primary human islets respond at a frequency of 72%³⁸. Although the percentage of NKX6.1⁺C-pep⁺ cells in their functional β -like cells is similar to that found in human islets, there were still some transcriptomic variations³⁸. Pagliuca et al. were successfully able to produce more β -like cells (33%) *in vitro* than other methods and showed that their β -like cells rescued diabetes in mice³⁸. This method offers promising clinical potential for type I diabetes; however, improvements in differentiation efficiency and the use of small molecules to replace growth factors for large-scale production are still necessary.

In 2016, the Melton group generated β -like cells from iPSCs derived from type I diabetic patients by using their previously developed protocol^{38,39}. The resulting β -like cells were monohormonal and they found 27% c-peptide⁺NKX6.1⁺ cells, which is similar to the 24% c-peptide⁺NKX6.1⁺ cells derived from iPSCs of non-diabetic patients³⁹. They found that their cells were able to generate insulin in response glucose changes both *in vitro* and *in vivo*, showing that the β -like cells they produced from diabetic patient donors had similar function to human β cells³⁹. However, they only received three type I diabetic donors, and therefore the implications of this study on the whole type I diabetic population is unknown³⁹. Additionally, as type I diabetes is an autoimmune disease, it might be expected that these β cells would provide minimal clinical efficacy without additional therapeutics to address the root cause of the disease.

Although the directed differentiation from hPSCs has shown promising results, there are still several challenges moving forward. The efficiency of producing functional mature β -like cells is low, and in order to achieve clinical application, efficient production of β cells in a cost-effective way will be crucial. Furthermore, high degrees of variance across hPSC lines indicate that current methods are not fully optimized. This could be related to evidence that there are multiple developmental pathways that can lead to a β cell fate⁴⁰. Once β cells are produced, quality control measures and standards also need to be developed to mitigate batch-to-batch variability. Additionally, the most efficient protocols to date have a high cost associated with low yield due to the heavy reliance on growth factors and serum (Table 1). Cost effective and chemically defined small molecule-based methods will provide increased translational potential.

Cellular reprogramming of other somatic cells into β -like cells

Cell fate and differentiation have classically been thought of as a branching pathway that once entered cannot be reversed or exchanged for a different one, meaning that cell fate is irreversible and non-plastic. Recent breakthroughs in the field, notably the advent of induced pluripotent stem cells in 2006^{2,3,41}, have definitively contradicted this belief and introduced new lines of investigation aimed at generating crucial cell types for therapeutic application. Transcription factor mediated cellular reprogramming is now a topic of great interest and has generated a large body of work aimed at reprogramming various somatic cell sources into, as is the focus of our discussion in this review, pancreatic β cells (Table 2).

Some of the initial efforts directed at somatic cell reprogramming focused on the use of other endodermal cells such as liver cells or pancreatic exocrine cells⁴². These first attempts investigated the use of transcription factors known to play important roles in pancreatic development, such as *Pdx1*, as reprogramming factors. In 2000, Ferber et al. showed that constitutive ectopic expression of *Pdx1* in mouse livers, via adenovirus, resulted in a significant increase in insulin expression and production in liver cells *in vivo*⁴². The group showed that the increased serum insulin levels helped to ameliorate the effects of streptozotocin-induced diabetes⁴². They do not, however, use any other measure aside from insulin production to show the generation of β cells nor do they claim to have generated β cells. The group does suggest that, due to the liver's natural regenerative capacity, there may be an abundance of multi-potent endodermal progenitor cells that are more responsive to transcription factor mediated activation of transdifferentiation gene networks⁴². In a similar study, adenovirus mediated delivery of stably expressed *Neurod1* and *Betacellulin* transgenes resulted in the generation of islet-like clusters of cells showing production of all major islet hormones⁴³. Further characterization of the resulting cell ultrastructure showed that potentially authentic insulin granules were present indicating native β cell processing and secretion of insulin in a glucose responsive manner⁴³. While the authors suggest islet neo-genesis is occurring, no definitive evidence was given to show the cellular origins of these induced β -like cells.

In 2008, Dr. Douglas Melton's group identified a group of three transcription factors, *Pdx1*, *Ngn3*, and *Mafa*, that they used to *in vivo* reprogram murine pancreatic exocrine cells to β cells⁴⁴. Lineage tracing was used to confirm the exocrine origins of the cells, an important determination in understanding cellular reprogramming, and extensive comparative experiments were conducted to show the resemblance of the induced β -like cells to native β cells⁴⁴. The induced cells were shown to resemble native β cell size, have insulin granules, express genetic markers of endocrine as opposed to exocrine cells, be monohormonal for insulin expression, and promote local angiogenesis⁴⁴. However, these cells did not form islet structures potentially hindering glucose responsiveness and highlighting the importance of microenvironment to cell function. Expanding on this work, Li et al. showed that increased reprogramming efficiency of acinar cells lead to long-term persistence of induced β cells in an *in vivo* mouse model⁴⁵. The use of polycistronic expression of the same three factors showed increased reprogramming efficiency resulting in the formation of islet like clusters of reprogrammed cells, allowing the long-term survival and developmental maturation of the cells⁴⁵. This allowed increased regulation of insulin secretion in response to glucose,

confirming the importance of the islet niche environment to the establishment of complete β cell function⁴⁵. *In vivo* cytokine mediated reprogramming of pancreatic acinar cells has also been demonstrated to restore normoglycemia after a period of chronic hyperglycemia in mice⁴⁶. This strategy activates similar signaling networks and genetic programs without the use of a viral vector, indicating potential efficacy of pharmacological agents for *in vivo* β cell reprogramming therapies.

Akinci et al. attempted to use the same factors for reprogramming of rat pancreatic exocrine cells *in vitro*⁴⁷. Interestingly, they observed an incomplete transformation of the cells highlighted in the lack of endogenous expression of transduced gene expression as well as genes critical to glucose responsiveness⁴⁷. This is likely attributed to the differences in the environmental cues present *in vivo* versus *in vitro*. Arguably, due to the clinical practice of donor islet transplantation, reprogramming strategies may be more readily translated to clinical use to achieve β cell reprogramming *in vitro* as opposed to *in vivo* where transcription factor transduction is less controlled. A recent study showed more effective *in vivo* reprogramming of murine antral stomach enteroendocrine cells than intestinal enteroendocrine cells by these three transcription factors as evidenced by molecular characterization of the β -like cell state⁴⁸. Furthermore, this study showed that the application of tissue engineering techniques allowed the transplantation of spheres of induced cells that are responsive to blood glucose levels⁴⁸. Interestingly, due to their highly regenerative cellular origins, these insulin positive cells regenerate upon ablation, an attractive characteristic when considering transplantation⁴⁸.

While murine models are useful tools, the translation to a human model system provides improved understanding of the therapeutic potential in cellular reprogramming techniques. As learned from early reprogramming efforts, somatic cells derived from common progenitor cell states are more easily reprogrammed to a β -like cell. This is largely attributed to the epigenetic landscape of the cell rendering the genes necessary for β cell identity more accessible. In a study conducted by Bar-Nur et al. iPSCs derived from human pancreatic β cells were shown to have increased propensity for differentiating to insulin positive cells both *in vitro* and *in vivo*⁴⁹. Even in the reprogrammed iPSC state, the β cell derived iPSCs showed an epigenetic signature more closely related to β cells than hPSCs with open chromatin structure at key β cell genes⁴⁹. This work demonstrates the importance of cell source in the development of not only reprogramming strategies, but also in directed differentiation. It also highlights the need to consider not just the gene expression programs that are being activated but also the changes that are being passively or actively made to the epigenetic landscape. Furthermore, a 2013 report showed that pancreatic α cells had both activating and repressing epigenetic factors localized to many genes expressed in β cells⁵⁰. They then showed that *in vitro* treatment with a histone methyltransferase inhibitor led to the expression of insulin and PDX1 in human and murine α cells⁵⁰. Most recently, Xiao et al. showed that adenovirus mediated *in vivo* conversion of murine α cells by expression of *Pdx1* and *Mafa* resulted in reprogrammed β cells that are very similar to actual β cells as determined by RNA sequencing⁵¹. However, these cells did not exhibit long-term survival in an autoimmune diabetic environment⁵¹. Similar to efforts in murine models, human acinar cells have been *in vitro* reprogrammed to β -like cells by viral infection with activated MAPK and STAT3, which were shown to activate expression of NGN3 and insulin⁵².

Interestingly, reprogramming efficiency in a 2D environment was lower than when a 3D Matrigel culture system was used, and further increases were seen upon engraftment into immune-compromised mice allowing maturation and acquired functionality⁵². These results reiterate the importance of environmental cues in determining the efficacy of cell reprogramming.

Due to the late developmental emergence of β cells, cellular reprogramming could provide a faster approach to generating mature β cells. Many of the reprogramming efforts have showed short-term efficacy on the scale of 1–2 weeks. However, many of these reprogramming strategies rely on integrating vectors that may not be suited for clinical translation. As such, refinement of these techniques to meet clinical standards will be required. Additionally, standards and ideal model systems for testing for efficacy and safety for *in vivo* reprogramming strategies will be needed as research advances into more therapeutically relevant models. Consideration and deeper understanding of the epigenetic landscape of mature β cells will also be important to creating an efficient reprogramming strategy and identifying an appropriate starting cell source. Alternatively, a fusion of *in vitro* reprogramming with tissue engineering may provide a faster route to therapeutic application due to a higher degree of control, but with retained concerns regarding immune response and cell source.

Clinical Trials and Translational Challenges

Currently, this field is moving towards clinical trials in patients with type 1 diabetes using hPSC derived pancreatic progenitor cells because restoring normal blood sugar levels in mice has been achieved previously by transplanting these cells³¹. The first patients received a transplant of these cells produced by ViaCyte in 2014 (Table 3). This initial phase of clinical investigation is focusing on the safety of the cell therapy combination products in patients. This strategy depends on the grafted pancreatic progenitors' spontaneous differentiation and maturation into mature β cells in the body. This study and two additional studies that are sponsored by Viacyte (Table 3) are the first and currently only clinical studies using hPSC derived cell products for diabetes therapy and results have not been published as of yet. An alternative approach is to directly use mature β cells derived from stem cells in clinical trials, testing their safety and efficacy for cell therapy, but no such trials currently exist. With the advent of recent success in generating more mature hPSC derived β cells from hPSCs³⁸, it is likely that phase 1 clinical trials based on using insulin-producing mature hPSC derived beta cells will soon follow.

For curing diabetes, generating functional mature β cells is one side of the coin. However, we need to acknowledge that avoiding attack from the immune system will be essential for treating type 1 diabetes and type 2 diabetes if allogeneic stem cell derived β cells are to be transplanted into patients. In 2016, scientists and engineers proposed to address this challenge by encapsulating functional β cells with alginate-derived porous polymer molecules⁵³. They tested their strategy in diabetic mice *in vivo* and found that they were able to control glucose levels without any immunosuppression⁵³. This strategy allows nutrient diffusion to the β cells but inhibits immune cell infiltration and attack⁵³. They have also initiated a possible strategy to deliver induced β cells into diabetic patients. An

alternative strategy to avoid allogeneic immune rejection might be to use immunoengineering approaches to remove proteins on the donor cell surface responsible for immune rejection or differentiate patient-specific iPSCs to β cells. However, in the case of type 1 diabetes, we still need to ask: how do we transplant patient-specific iPSC derived β cells into the same patient that has a specific immune reaction to their own beta cells.

Concluding Remarks and Future Directions

In summary, tremendous progress has been made toward both understanding β cell and pancreas development *in vivo* during normal development and how to make functional β cells *in vitro* through stem cell differentiation and cellular reprogramming. We need to acknowledge, though, that the challenge of generating sufficient bona fide β cells *in vitro* with high conversion efficiency (> 90%, similar to what we have achieved in cardiomyocyte differentiation^{5–7}) for cost effective therapeutic application remains unmet. Currently the most efficient protocol for generating mature β -like cell has a yield of 33% and relies heavily on expensive growth factors making it more difficult to translate to clinical application. Additionally, variability in batches or between cell lines is another barrier to clinical application as well as to optimization of differentiation methods. Furthermore, differentiation time remains a challenge when mimicking developmental emergence of pancreatic β cells. Reprogramming strategies have been shown to reduce the time required to generate β cells; however, many of the strategies rely on integrating factors that are not ideal for clinical application and the epigenetic landscape of the source cell could have unknown effects on the reprogrammed cell type.

Once mature β cells can be obtained efficiently, immune rejection of transplanted cells remains a primary concern. This challenge is currently stimulating collaborative research to determine how β cells might be protected from immune rejection and still receive nutrients and function normally. In spite of the barriers that the field still faces in differentiation and reprogramming to obtain mature β cells, some progenitor cells are being tested in clinical trials as an intervention for type 1 diabetes. Due to the increasing health threat proposed by diabetes, it is reasonable to expect that additional innovations regarding and clinical trials using stem cell derived or reprogrammed β cells are soon to follow.

References

1. Thomson JA et al. Embryonic stem cell lines derived from human blastocysts. *Science* 282, 1145–7 (1998). [PubMed: 9804556]
2. Takahashi K & Yamanaka S Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. *Cell* 126, 663–676 (2006). [PubMed: 16904174]
3. Yamanaka S et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131, 861–72 (2007). [PubMed: 18035408]
4. Yu J et al. Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells. *Science* (80-.). 318, 1917 LP–1920 (2007). [PubMed: 18029452]
5. Lian X et al. Robust cardiomyocyte differentiation from human pluripotent stem cells via temporal modulation of canonical Wnt signaling. *Proc. Natl. Acad. Sci* 109, E1848–E1857 (2012). [PubMed: 22645348]
6. Lian X et al. Chemically defined, albumin-free human cardiomyocyte generation. *Nat. Methods* 12, 595–596 (2015). [PubMed: 26125590]

7. Lian X et al. Directed cardiomyocyte differentiation from human pluripotent stem cells by modulating Wnt/ β -catenin signaling under fully defined conditions. *Nat. Protoc* 8, 162–75 (2013). [PubMed: 23257984]
8. Benitez CM, Goodyer WR & Kim SK Deconstructing pancreas developmental biology. *Cold Spring Harb. Perspect. Biol* 4, 1–17 (2012).
9. Dunning BE, Foley JE & Ahren B Alpha cell function in health and disease: influence of glucagon-like peptide-1. *Diabetologia* 48, 1700–1713 (2005). [PubMed: 16132964]
10. Hauge-evans AC et al. Somatostatin Secreted by Islet δ -Cells Fulfills Multiple Roles as a Paracrine Regulator of Islet Function 58, 403–411 (2009).
11. Batterham RL et al. Pancreatic polypeptide reduces appetite and food intake in humans. *J. Clin. Endocrinol. Metab* 88, 3989–3992 (2003). [PubMed: 12915697]
12. Dickson SL et al. The role of the central ghrelin system in reward from food and chemical drugs. *Mol. Cell. Endocrinol* 340, 80–87 (2011). [PubMed: 21354264]
13. Hou JC, Min L & Pessin JE Insulin Granule Biogenesis, Trafficking and Exocytosis. *Vitam Horm* 478–506 (2009). doi:10.1016/S0083-6729(08)00616-X. Insulin
14. Yoon J-W & Jun H-S Autoimmune Destruction of Pancreatic β Cells: American Journal of Therapeutics. *Am. J. Ther* 591, 580–591 (2005).
15. Borchers AT, Uibo R & Gershwin ME Autoimmunity Reviews The geoepidemiology of type 1 diabetes. *Autoimmun. Rev* 9, A355–A365 (2018).
16. Sander M & German MS The beta cell transcription factors and development of the pancreas. *J. Mol. Med. (Berl)* 75, 327–40 (1997). [PubMed: 9181474]
17. Landsberg L & Molitch M Diabetes and Hypertension: Pathogenesis , Prevention and Treatment y 26, 621–628 (2004).
18. Tao B, Pietropaolo M, Atkinson M, Schatz D & Taylor D Estimating the Cost of Type 1 Diabetes in the U . S . : A Propensity Score Matching Method 5, (2010).
19. Pan FC & Wright C Pancreas organogenesis: From bud to plexus to gland. *Dev. Dyn* 240, 530–565 (2011). [PubMed: 21337462]
20. Dhanantwari P et al. Human Cardiac Development in the First Trimester A High-Resolution Magnetic Resonance Imaging and Episcopic Fluorescence Image Capture Atlas. *Circulation* 343–351 (2009). doi:10.1161/CIRCULATIONAHA.108.796698 [PubMed: 19635979]
21. Pagliuca FW & Melton DA How to make a functional beta cell. *Development* 140, 2472–2483 (2013). [PubMed: 23715541]
22. Gannon M, Herrera P & Wright CV E. Mosaic Cre-Mediated Recombination in Pancreas Using the pdx-1 Enhancer / Promoter. *Genesis* 144, 143–144 (2000).
23. Kim SK & Melton DA Pancreas development is promoted by cyclopamine , a Hedgehog signaling inhibitor. *Proc. Natl. Acad. Sci* 95, 13036–13041 (1998). [PubMed: 9789036]
24. Molotkov A, Molotkova N & Duester G Retinoic Acid Generated by Raldh2 in Mesoderm Is Required for Mouse Dorsal Endodermal Pancreas Development. *Dev. Dyn* 950–957 (2005). doi: 10.1002/dvdy.20256 [PubMed: 15739227]
25. Apelqvist A, et al. Notch signalling controls pancreatic cell differentiation. *Nature* 400, 877–881 (1999). [PubMed: 10476967]
26. Lumelsky N et al. Differentiation of Embryonic Stem Cells to Insulin-Secreting Structures Similar to Pancreatic Islets. *Science* (80-.). 292, 1389 LP–1394 (2001). [PubMed: 11326082]
27. Otonkoski T, Beattie GM, Mally MI, Ricordi C & Hayek A Nicotinamide is a potent inducer of endocrine differentiation in cultured human fetal pancreatic cells. *J. Clin. Invest* 92, 1459–1466 (1993). [PubMed: 8104197]
28. Cogger KF et al. Glycoprotein 2 is a specific cell surface marker of human pancreatic progenitors. *Nat. Commun.* (2017). doi:10.1038/s41467-017-00561-0
29. D'Amour KA et al. Efficient differentiation of human embryonic stem cells to definitive endoderm. *Nat. Biotechnol* 23, 1534–1541 (2005). [PubMed: 16258519]
30. D'Amour KA et al. Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. *Nat. Biotechnol* 24, 1392–1401 (2006). [PubMed: 17053790]

31. Kroon E et al. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. *Nat. Biotechnol* 26, 443– 452 (2008). [PubMed: 18288110]
32. Jiang J et al. Generation of Insulin-Producing Islet-Like Clusters from Human Embryonic Stem Cells. *Stem Cells* 25, 1940–1953 (2007). [PubMed: 17510217]
33. Nostro MC et al. Stage-specific signaling through TGF β family members and WNT regulates patterning and pancreatic specification of human pluripotent stem cells. *Development* 138, 861–871 (2011). [PubMed: 21270052]
34. Inman GJ et al. SB-431542 Is a Potent and Specific Inhibitor of Transforming Growth Factor- β Superfamily Type I Activin Receptor-Like Kinase (ALK) Receptors ALK4, ALK5, and ALK7. *Mol. Pharmacol* 62, 65–74 (2002). [PubMed: 12065756]
35. Nostro MC et al. Efficient generation of NKX6–1+ pancreatic progenitors from multiple human pluripotent stem cell lines. *Stem Cell Reports* 4, 591–604 (2015). [PubMed: 25843049]
36. Herrera PL Adult insulin- and glucagon-producing cells differentiate from two independent cell lineages. *Development* 2322, 2317–2322 (2000).
37. Rezanian A et al. Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells 32, (2014).
38. Pagliuca FW et al. Generation of functional human pancreatic β cells in vitro. *Cell* 159, 428–439 (2014). [PubMed: 25303535]
39. Millman JR et al. Generation of stem cell-derived β -cells from patients with type 1 diabetes. *Nat. Commun* 7, (2016).
40. Petersen MBK et al. Single-Cell Gene Expression Analysis of a Human ESC Model of Pancreatic Endocrine Development Reveals Different Paths to β -Cell Differentiation. *Stem Cell Reports* 9, 1246–1261 (2017). [PubMed: 28919263]
41. Okita K, Ichisaka T & Yamanaka S Generation of germline-competent induced pluripotent stem cells. *Nature* 448, 313–317 (2007). [PubMed: 17554338]
42. Ferber S et al. Pancreatic and duodenal homeobox gene 1 induces expression of insulin genes in liver and ameliorates streptozotocin-induced hyperglycemia. *Nat. Med* 6, 568– 572 (2000). [PubMed: 10802714]
43. Kojima H et al. NeuroD-betacellulin gene therapy induces islet neogenesis in the liver and reverses diabetes in mice. *Nat. Med* 9, 596–603 (2003). [PubMed: 12704384]
44. Zhou Q, Brown J, Kanarek A, Rajagopal J & Melton DA In vivo reprogramming of adult pancreatic exocrine cells to β -cells. *Nature* 455, 627–632 (2008). [PubMed: 18754011]
45. Li W et al. Long-term persistence and development of induced pancreatic beta cells generated by lineage conversion of acinar cells. *Nat. Biotechnol* 32, 1223–1230 (2014). [PubMed: 25402613]
46. Baeyens L et al. Transient cytokine treatment induces acinar cell reprogramming and regenerates functional beta cell mass in diabetic mice. *Nat. Biotechnol* 32, 76–83 (2014). [PubMed: 24240391]
47. Akinci E, Banga A, Greder LV, Dutton JR & Slack JMW Reprogramming of pancreatic exocrine cells towards a beta (β) cell character using *Pdx1* , *Ngn3* and *MafA*. *Biochem. J* 442, 539–550 (2012). [PubMed: 22150363]
48. Ariyachet C et al. Reprogrammed Stomach Tissue as a Renewable Source of Functional β Cells for Blood Glucose Regulation. *Cell Stem Cell* 18, 410–421 (2016). [PubMed: 26908146]
49. Bar-Nur O, Russ HA, Efrat S & Benvenisty N Epigenetic memory and preferential lineage-specific differentiation in induced pluripotent stem cells derived from human pancreatic islet beta cells. *Cell Stem Cell* 9, 17–23 (2011). [PubMed: 21726830]
50. Bramswig NC et al. Epigenomic plasticity enables human pancreatic alpha to beta cell reprogramming. *J Clin Invest* 123, 1275–1284 (2013). [PubMed: 23434589]
51. Xiao X et al. Endogenous Reprogramming of Alpha Cells into Beta, Induced by Viral Gene Therapy , Reverses Autoimmune Diabetes. *Cell Stem Cell* 22, 78–90 (2018). [PubMed: 29304344]
52. Lemper M et al. Reprogramming of human pancreatic exocrine cells to β -like cells. *Cell Death Differ* 22, 1117–1130 (2015). [PubMed: 25476775]
53. Vegas AJ et al. Long-term glycemic control using polymer-encapsulated human stem cell-derived beta cells in immune-competent mice. *Nat. Med* 22, 306–311 (2016). [PubMed: 26808346]

Fig 1.
Schematic of beta cell functions.

Nostro et al, Stem Cell Reports, 2015

Pagliuca et al, Cell, 2014

Fig 2.
Schematic of two beta cell differentiation protocols.

Table 1:

Summary of all directed differentiation strategies.

GROUP/ YEAR	TECHNIQUE	RESULTS	WEAKNESS
Lumelsky et al. 2001 ²⁶	<i>in vitro</i>	50X less insulin production than human β cells	did not follow developmental pathway
D'Amour et al. 2005 ²⁹	<i>in vitro</i>	80% SOX17 ⁺	variance across cell lines
D'Amour et al. 2006 ³⁰	<i>in vitro</i>	process 64% of proinsulin content	produced immature fetal β cells
Kroon et al. 2008 ³¹	<i>in vivo</i>	92% of implanted mice produced maximal insulin secretion	teratoma formation from undifferentiated cells
Jiang et al. 2007 ³²	<i>in vitro</i>	64.9% SOX17 ⁺ /15–25% PDX1 ⁺	problems with endocrine specification
Nostro et al. 2011 ³³	<i>in vitro</i>	25% C-peptide ⁺	low efficiency
Nostro et al. 2015 ³⁵	<i>in vitro</i>	70% NKX6.1 ⁺	cell line dependent
Rezania et al. 2014 ³⁷	<i>in vitro</i> <i>in vivo</i>	50% insulin ⁺ /PDX1 ⁺ /NKX6.1 ⁺ reversal of diabetes in 6 weeks	functionally immature β cells
Pagliuca et al. 2014 ³⁸	<i>in vitro</i>	75% response to glucose treatment	transcriptomic variation
Millman et al. 2016 ³⁹	<i>in vitro</i>	27% monohormonal C-peptide ⁺ /NKX6.1 ⁺	based on 3 diabetic patients

Table 2:

Summary of all reprogramming strategies.

GROUP/ YEAR	TECHNIQUE	STRATEGY	RESULTS	WEAKNESS
Ferber et al. 2000 ⁴²	<i>in vivo</i>	adenovirus of PDX1	increase insulin production	did not generate β cells
Kojima et al. 2003 ⁴³	<i>in vitro</i>	adenovirus of NEUROD1 and betacellulin	hormonal islet-like clusters	no evidence to show cellular origins
Melton et al. 2008 ⁴⁴	<i>in vivo</i>	adenovirus	induced β cell with proper size, insulin granules, and genetic markers	no islet formation
Li et al. 2014 ⁴⁵	<i>in vivo</i>	polycistronic coexpression of adenovirus of Ngn3, Pdx1, and Mafa	islet-like clusters increased reprogramming efficiency	experiments conducted in mice, not human
Baeyens et al. 2014 ⁴⁶	<i>in vivo</i>	treatment with epidermal growth factor (EGF) and ciliary neurotrophic factor (CNTF)	restore normoglycemia in chronic hyperglycemic mice	experiments conducted in mice, not human
Akinci et al. 2012 ⁴⁷	<i>in vitro</i>	adenovirus of NGN3, PDX1, MAFA	reprogrammed cells have 3% of normal insulin content	incomplete transformation
Ariyachet et al. 2016 ⁴⁸	<i>in vivo</i>	adenovirus of NGN3, PDX1, MAFA	found conversion of murine antral stomach enteroendocrine to β cells more effective	experiments conducted in mice, not human
Bramswig et al. 2013 ⁵⁰	<i>in vitro</i>	used histone methylase inhibitor	insulin ⁺ / PDX1 ⁺ in human and mouse α cells	results in α cells, not β cells
Xiao et al. 2018 ⁵¹	<i>in vivo</i>	adenovirus of PDX1 and MAFA	converted murine α cells to β cells	no long-term survival in autoimmune diabetic environment
Lemper et al. 2015 ⁵²	<i>in vitro</i>	virus with MAPK and STAT3	NGN3 ⁺ / insulin ⁺	using virus could cause gene insertion at improper sites

Table 3:

Summary of all clinical trials using stem cell derived pancreatic β cells or progenitors.

NAME	START DATE	STATUS	SPONSOR	LOCATION	PHASE
A Safety, Tolerability, and Efficacy Study of VC-01™ Combination Product in Subjects With Type 1 Diabetes Mellitus	September 2014	Active, not recruiting	Viacyte	USA and Canada	Phase 1 Phase 2
A Safety and Tolerability Study of VC-02™ Combination Product in Subjects With Type 1 Diabetes Mellitus	July 5, 2017	Active, not recruiting	Viacyte	Canada	Phase 1
A Safety, Tolerability, and Efficacy Study of VC-02™ Combination Product in Subjects With Type 1 Diabetes Mellitus and Hypoglycemia Unawareness	July 6, 2017	Recruiting	Viacyte	USA and Canada	Phase 1 Phase 2