Recent Advances within iPSC-Derived Beta Cell Chemical Differentiation Protocols

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Diabetes is a condition that affects many people worldwide. By studying beta cells, which produce insulin and are lost in diabetes, researchers can gain insight into disease progression and potential treatments. Researchers have developed protocols to culture these cells more efficiently and make them accurate for human beta cells. This literature review explores the recent advancements of iPSC to beta cell differentiation within the chemical differentiation protocols. Specifically, growth factors such as Activin A and EGF assist glucose-stimulated insulin secretion (GSIS) within the in-vitro cultured cells. Other small molecule modulators like ERRγ agonists, Notch-signaling pathway inhibitors, and TGF-beta inhibitors are essential in regulating signaling pathways, crucial in iPSC to beta cell differentiation. Growth conditions like hypoxia and 3D culturing can help simulate the in-vivo cell environment. Researchers continue exploring different methods of culturing beta cells in vitro to improve differentiation efficiency, study diabetes progression within beta cells, and use them in novel cell replacement therapies for diabetes.

Introduction

Diabetes is a chronic disease that impairs the body's ability to regulate blood sugar. As of 2021, it affected approximately 38.4 million adults in the U.S., including 2 million with type 1 diabetes[1]. Chronic diabetes is categorized into two main types: Type 1 and Type 2. Type 1 diabetes is an autoimmune disorder in which the body's immune system attacks insulin-producing beta cells in the pancreas. This makes it difficult for the body's cells to absorb glucose from the bloodstream to use as energy, leading to high blood sugar levels, which can result in various complications[2]. Since there is no available cure for type 1 diabetes, treatment requires lifelong insulin therapy, either through injections or an insulin pump. However, these therapies carry the risk of administering too much insulin, which can lead to dangerously low blood sugar levels, a condition known as iatrogenic hypoglycemia[2].

Meanwhile, in type 2 diabetes, the body cannot properly utilize insulin due to insulin resistance in peripheral tissues. Initially, beta cells compensate by producing more insulin, but over time, glucose-stimulated insulin secretion (GSIS) becomes impaired, ultimately leading to beta cell loss[3]. This increased strain on the remaining beta cells eventually causes further loss due to cell exhaustion. Currently, the available therapies for type 2 diabetes include changes in diet, exercise, and usage of medication such as metformin and glinides to control blood sugar levels. Both types of diabetes can lead to an increased risk of other health complications such as cardiovascular disease, diabetic neuropathy, and kidney damage[3]. Diabetes has multiple variations and affects a large proportion of the population. Current treatments for diabetes stabilize the condition and reduce complications but can have side effects and do not have the ability to completely cure the disease. These limitations highlight the need for continued research into more effective treatments. Modelling the disease within beta cells offers insights and avenues for therapy development.

Conventionally, mice have been crucial to understanding diabetes and finding potential treatments and therapies. Using mice models, such as nonobese diabetic (NOD) mice, as a medium to study human diabetes progression has led to significant breakthroughs in understanding the immune response underlying Type 1 diabetes and has been crucial to exploring target immunotherapy to help type 1 diabetes patients[4]. However, recent studies, such as Reed and Herold (2015)[5], mentioned the differences in disease progression between humans and mice due to the differences between mouse and human physiology. Their paper highlights certain limitations in applying mice model data to human diabetes. For instance, they reference Mestas and Hugh et al. (2004), who state that interferons' function, which plays a crucial role in the immune response against beta cells in type 1 diabetes, differs between NOD mice and humans. In humans, interferons activate a type 1 T helper (TH1) cell response in immune cells, whereas this does not occur in mice[6].

One alternative for studying beta cell development has been using human pancreatic biopsies and cadaver pancreases to extract cells to observe beta cell function and diabetes. These were considered to have certain advantages over mouse models, as they better reflect human disease progression due to the similarities in physiology compared to mice, particularly in insulitis, where immune cells infiltrate the pancreas, a characteristic of

type 1 diabetes. However, both are in limited supply, making it challenging to observe human pancreatic development and test potential diabetes treatments through them[7]. Researchers then turned to human pluripotent stem cells (hPSCs) as a new source for studying beta cell development to overcome the limited availability of human pancreatic beta cells. HPSCs have been shown to differentiate into various forms of specialized cells in all three germ layers. There are two main types of human pluripotent stem cells (hPSCs) used in research: human embryonic stem cells (hESCs), which are often avoided due to ethical concerns, and induced pluripotent stem cells (iPSCs), which are created by reprogramming human adult somatic cells into stem cells. The pioneering work on iPSCs was conducted by Takahashi et al. (2007), who introduced four key factors—Oct3/4, Sox2, Klf4, and c-Myc—into human fibroblasts using a retrovirus. Oct3/4 and Sox2 are essential for maintaining pluripotency by suppressing differentiation-related transcription factors. However, their binding is inhibited by epigenetic barriers such as DNA methylation. Klf4 and c-Myc were employed to help overcome these barriers, allowing Oct3/4 and Sox2 to bind and initiate reprogramming, transforming the cell into a pluripotent state[8]. Researchers are developing and refining protocols for differentiating iPSCs into mature beta cells to address the shortage of available beta cells for research. Producing cells that closely resemble mature beta cells is crucial for simulating beta cell development and studying the progression of diabetes, including both type 1 and type 2. This approach also holds the potential for developing beta cell-based treatments for diabetes. By comparing and analyzing the iPSC to beta cell differentiation protocols, researchers can gain valuable insights into existing challenges and inefficiencies, which could be targeted for optimization to improve efficiency and differentiate cells most closely resembling human pancreatic beta cells. These protocols consider the process of differentiation of iPSCs into beta cells via a chemical differentiation protocol, including the maturity, viability, efficiency, and uniformity of the resulting beta cells. Since these protocols are primarily conducted in vitro, there is a need to replicate the conditions present in vivo to identify essential molecules, pathways, and culturing conditions to attain mature beta cells efficiently.

Methods

This literature review examines the key components of protocols for differentiating induced pluripotent stem cells (iPSCs) and foundational studies showcasing the function of those components in embryonic stem cell-based protocols, which can be applied to iPSCs. The search strategy involved using databases like PubMed and Google Scholar with keywords such as *induced pluripotent stem cells, beta cell differentiation, diabetes, chemical differentiation protocols, growth factors, culture conditions, and small molecule modulators*. The review specifically targets components like growth factors (e.g., Activin A, CHIR99021, EGF), small molecules (e.g., TGF-beta inhibitor Alk5i), and culture conditions (e.g., hypoxia and 3D culturing).

I began by referencing Fantuzzi et al. (2022) and Velazco-Cruz et al. (2019)[9] to identify the most up-to-date chemical differentiation protocols for converting human pluripotent stem cells (hPSCs) into beta cells. These protocols included the aforementioned key components. To trace the evolution of these methodologies and understand how recent modifications improved outcomes, I explored earlier studies cited in these papers, leading to protocols such as Pagliuca et al. and foundational works like D'Amour et al. (2005). I referenced twenty sources from Pubmed, ten sources from Google Scholar, and other sources from diabetes databases. I restricted the papers to the papers with the most citations referenced within the most recent protocols, i.e., Pagliuca et al. (2014), Fantuzzi et al. (2022), Velazco-Cruz et al. (2019), Zhang et al. (2009), and documents explicitly looking at a component mentioned above.

A potential limitation of this review is the inability to access research that has not been published publicly or was being conducted at the time of the writing of this paper. These constraints may limit access to more recent developments in the field. Additionally, I did not have access to a lab to directly compare the protocols for their efficiency and similarities in their results.

Beta Cell Development

Protocols for in vitro differentiation of induced pluripotent stem cells (iPSCs) into beta cells often aim to mimic the stages of normal embryonic pancreatic development. In humans, the pancreas arises from two dorsal and ventral buds originating from the foregut endoderm (Fig. 1A). This process begins around 26–31 days post-fertilization. By week 5 of embryonic development, the rotation of the stomach shifts the ventral pancreatic bud behind the dorsal bud (Fig. 1B)[10]. As development progresses, the ductal systems of these buds fuse, eventually forming a single organ[11]. The ventral bud gives rise to the head of the pancreas, while the dorsal bud forms the bulk of the final fused organ[10]. Finally, the pancreas connects with the duodenum and common bile duct, facilitating exocrine secretion into the digestive tract (Fig. 1C).

As the pancreas develops, it gives rise to both endocrine and exocrine cells. The exocrine pancreas, primarily composed of acinar and ductal cells, produces and transports secretions such as digestive enzymes to aid in digestion[14]. The endocrine portion, known as the islets of Langerhans, consists of clusters of specialized cells, including alpha, beta, delta, epsilon, and pancreatic polypeptide cells. These cells secrete hormones into the bloodstream, including glucagon from alpha cells, insulin from beta cells, somatostatin from delta cells, ghrelin from epsilon cells, and pancreatic polypeptide from pancreatic polypeptide cells[15].

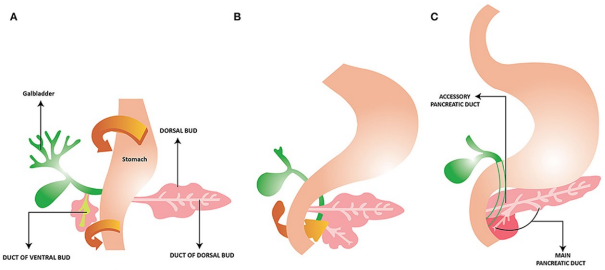

Fig. 1 Key stages of pancreatic organogenesis in a pictorial format. This image is referenced from Mehta et al. (2022)[12], who adopted the image from Ojo A. S. (2020)[13]

Transcription Factors

Within the process of pancreatic development, transcription factors play an important role in directing cell fate from stem cells into mature beta cells. Three of the main transcription factors are Neurogenin-3, Pancreatic duodenal homeobox-1, and Mast cell function-associated antigen. Neurogenin-3 (NGN3) is a key regulator during pancreatic organogenesis, guiding the differentiation of progenitor cells into endocrine lineages. It is pivotal in activating transcription factors like NeuroD1 and Pax4, which are crucial for endocrine cell development and function[16]. Gradwohl et al. (2000) demonstrated NGN3's necessity for forming endocrine cell types, as knockout mice lacked mature endocrine cells but not necessarily all early precursor stages. In NGN3 knockout mice, a failure to develop any endocrine cells led to elevated blood glucose levels, highlighting its importance in beta cell differentiation and insulin regulation[17]. NGN3 is highly expressed during the endocrine precursor stage but not in mature insulin- or glucagon-expressing cells. Next, Pancreatic duodenal homeobox-1 (PDX1) is expressed early during the differentiation of the foregut endoderm into the ventral and dorsal pancreatic buds[18]. It activates NGN3 in conjunction with HNF6, another transcription factor important for pancreatic organogenesis[19]. Holland et al. (2002) demonstrated that inhibiting PDX1 expression with doxycycline in pregnant mice prevented the development of acinar and islet tissues in the offspring's pancreas, highlighting the importance of PDX1 expression during the developmental stages of the pancreas[20]. PDX1 regulates insulin gene expression and is crucial for maintaining beta cell identity by controlling glucose-sensing genes that enable insulin secretion. Mast cell function-associated antigen (MAFA) can upregulate PDX1, but it is not essential for its expression[19]. MAFA plays a crucial role in enhancing the expression of several genes involved in glucose-stimulated insulin secretion (GSIS), a key characteristic of mature beta cells. These genes include *Ins1, Slc2a2, Slc30a8, Pcsk1*[21]. MAFA plays a crucial role in maintaining beta cell identity; its absence can lead mature beta cells to revert to a precursor state, resulting in the loss of the insulin-producing identity of a beta cell[21]. MAFA, PDX1, and NGN3 are seen to be essential in human beta cell development and are thus used by researchers to assess the stages of differentiation progression within in-vitro protocols such as chemical differentiation.

Chemical Differentiation

Chemical differentiation protocols guide the in vitro differentiation of human pluripotent stem cells (hPSCs) into mature somatic cells by using small molecules, signaling pathway inhibitors, and growth factors. In the case of chemical differentiation with the goal of creating a mature beta-like cell, the process typically starts with hPSCs, progresses through intermediate stages, and culminates in the formation of mature beta-like cells. Although the number of stages varies across beta cell differentiation protocols such as with Fantuzzi et al. (2022) and Zhang et al. (2009), which contain 7 and 5 stages, respectively, which may arise from differences in the control over component addition at various stages, but generally encompass five main phases: hPSCs, definitive endoderm, pancreatic progenitor, endocrine progenitor, and mature beta-like cells (Fig. 2). When using iPSCs, the process begins with the reprogramming of mature human fibroblasts into iPSCs by the exogenous expression of four transcription factors: Oct3/4, Sox2, Klf4, and c-Myc[8]. The next step in differentiation is the definitive endoderm stage, which can give rise to various cell types within the gastrointestinal and respiratory tracts. Sox17 is commonly used as a marker for this stage in many studies[22,23]. Next, the endocrine progenitor, a differentiated form of pancreatic progenitor, facilitates further differentiation into mature endocrine cells. The final differentiation step is the generation of mature beta-like cells. The final differentiated cell is considered to be a beta-like cell if it can secrete insulin in response to glucose, performing GSIS.

Protocol Components

When designing protocols for differentiating iPSCs into beta-like cells, key components that are considered are growth factors, small molecular modulators, and culture conditions. Firstly, growth factors are crucial in maintaining the cells' ability to differentiate and specialize into beta cells. A common growth factor in earlier protocols, like those by Pagliuca et al. (2014)[26] and more recent studies like Fantuzzi et al. (2022), is Activin A, which is introduced in the initial stages following hPSC differentiation. Activin A supports the expression of pluripotency and aids in the transition toward the pancreatic progenitor cells, but its primary role is to direct cells toward the endoderm stage. D'Amour et al. (2005), a protocol testing definitive endoderm differentiation within embryonic stem cells, showed that Activin A can also promote differentiation into the definitive endoderm

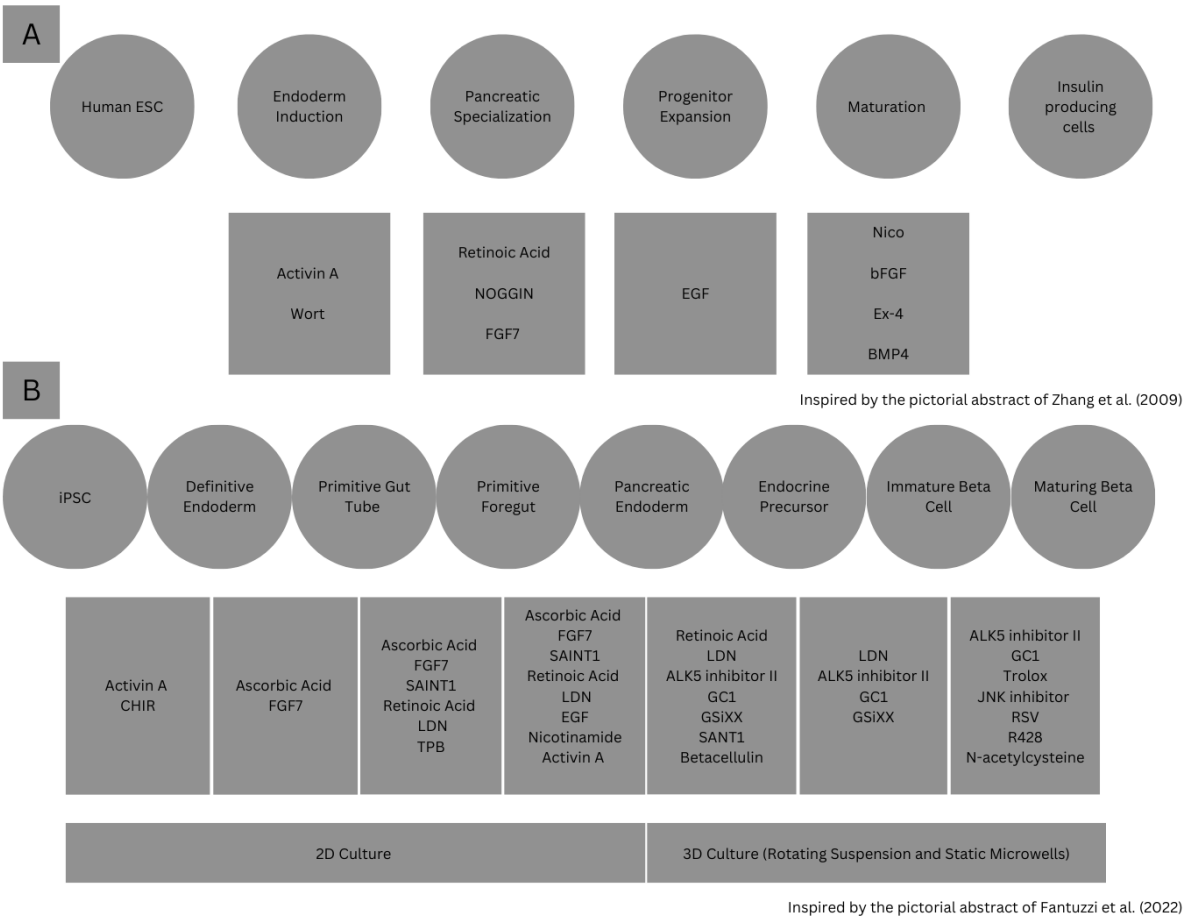

Fig. 2 (A) Zhang et al. (2009)[24] follow a five-stage protocol, utilizing distinct media formulations at each stage. **(B)** Fantuzzi et al. (2022)[25] follow a seven-stage protocol, which includes specific steps for basal media with various chemicals and incorporates a switch from 2D to 3D culturing methods over a period of approximately 30 days.

phase by upregulating transcription factors such as Sox17 and FoxA2, which are markers associated with beta cell development[27]. SRY-box transcription factor 17(Sox17) is crucial to directing the cell into the definitive endoderm. Thus, overexpression of Sox17 is introduced during the mesoderm stage, resulting in high expression of other definitive endoderm markers such as FOXA2, GSC, GATA4, and HEX[28]. While Activin A is beneficial in differentiation protocols within promoting the definitive endoderm stage, its effects depend on the dosage used in the initial stages. Tomizawa et al. (2011)[29] demonstrated that stem cells treated with 100 ng/ml of Activin A maintained their pluripotency compared to controls with 0 ng/ml. They identified undifferentiated cells by staining for markers like Oct3/4 and Nanog, noting that exposure to 10 ng/ml of Activin A showed positive staining. Their findings indicated that Activin A at concentrations of 3-30 ng/ml promotes cell proliferation, while 100 ng/ml suppresses it. In protocols such as those by Pagliuca et al. (2014) and Fantuzzi et al. (2022), Activin A is utilized to differentiate iPSCs into the definitive endoderm phase. An important modification to earlier protocols, like that of D'Amour et al. (2005), includes the addition of CHIR99021, a GSK-3 inhibitor that enhances iPSC proliferation into the definitive endoderm. Kunisada et al. (2012) found that CHIR99021 promotes differentiation by activating the Wnt signaling pathway, which is crucial for mesodermal and definitive endoderm formation[30].

Epidermal Growth Factor (EGF) is another commonly used growth factor that enhances cell proliferation during the pancreatic progenitor stage. Zhang et al. (2009) incorporated EGF during the progenitor expansion stage to promote beta cell maturation. They found that EGF-treated PDX1-positive cells exhibited increased PDX1 expression, with 38.6% of cells expressing it compared to only 12.8% in non-EGF-exposed PDX1-positive cells. This indicates that over-expression of EGF within cells could aid in expanding and encouraging proliferation within pancreatic progenitor cells. Additionally, EGF treatment resulted in higher expression of Ki67, a proliferation marker, supporting

enhanced beta cell proliferation. Other markers indicative of pancreatic progenitor cells, such as PDX1, FOXA2, SOX9, and HNF1B, were also upregulated in EGF-treated cells. This inclusion of EGF in differentiation protocols contributed to increased expression of insulin and PDX1, as well as MAFA for beta cell maturity. Fantuzzi et al. (2022) used EGF in combination with retinoic acid and KGF. Keratinocyte growth factor (KGF), also known as fibroblast growth factor- 7(FGF7), is used to aid in the proliferation and expansion of pancreatic progenitor cells. Retinoic acid has been used in protocols to aid in expressing PDX1, an essential transcription factor in pancreatic development, to direct cell fate into becoming a pancreatic progenitor. When used together, Pagliuca et al. (2014) demonstrated that they expressed NKX6-1, which is essential for differentiating cells into the pancreatic progenitor stage, and PDX1. Fantuzzi et al. (2022) likely used EGF to support the proliferation of the pancreatic progenitors, which were defined by their expression of PDX1 and NKX6-1.

Small molecules are crucial in regulating signaling pathways during the differentiation from iPSCs to beta cells. Velazco-Cruz et al. (2019) emphasized the role of TGF-beta signaling in GSIS in beta cells. They introduced Alk5i after pancreatic progenitors transformed into endocrine precursor cells to inhibit TGF-beta signaling. Their findings indicated that treating mature beta cells with Alk5i negatively impacted insulin secretion. On the contrary, inhibiting TGF-beta signalling during the endocrine induction stage improved insulin secretion in mature beta-like cells, enabling pulsatile insulin release. This was seen as an advancement as a crucial element that earlier protocols like Pagliuca et al. (2014) and D'Amour et al. (2005) highlighted the inefficiency of pulsatile insulin release in response to glucose, an essential characteristic of functional beta cells within the beta-like cells which they differentiated. Within humans, the pulsatile behavior of beta cell release occurs in two phases: ultradian oscillation, triggered by glucose detection, lasting about 1-2 hours, and rapid oscillation, which lasts 1-15 minutes. This multi-phase insulin release is believed to help the pancreas maintain glucose levels more effectively by releasing insulin in pulses rather than continuously[31]. The demonstration of the pulsatile behavior in the beta cells generated by Velazco-Cruz et al. (2019) demonstrated that the placement of Activin A within the protocol has a significant impact on the differentiation and demonstrated the pulsatile behavior within in-vitro generated beta-like cells. Additionally, the TGF-beta pathway has also been shown to promote the expression of Smad7, which inhibits beta cell proliferation[32]. Some studies have leveraged this knowledge to enhance beta cell proliferation by blocking the TGF-beta signaling pathway. This inhibition counteracts Smad7's negative effects, promoting MAFA expression and contributing positively to the maturation of the differentiated beta cells[33].

Recent application of ERRγ within hPSC to beta cell differentiation protocols has indicated its role in beta cell maturation by the role of metabolic regulation. Jin and Jiang emphasize metabolic maturation to enhance the ability of generated cells to perform glucose-stimulated insulin secretion (GSIS), addressing the inefficiencies observed in earlier protocols. ERRγ (estrogen-related receptor γ) is believed to regulate cell metabolism, particularly mitochondrial function[34]. Yoshihara et al. (2016)[35] demonstrated the importance of ERRγ in GSIS using βERRγKO mice, which lack functional ERRγ and fail to increase insulin secretion in response to glucose, unlike wild-type (WT) and WT(RIP-Cre) mice. This suggests that ERRγ is crucial for beta cell maturity and regulating mitochondrial pathways necessary for GSIS. By overexpressing ERRγ in iPSC-derived beta cells, they observed enhanced c-peptide secretion, indicating improved insulin production upon glucose exposure. Jin and Jiang (2022) tested the overexpressing ERRγ during the final stages to promote the metabolic maturation of beta-like cells for effective GSIS. More recently, Nair et al. (2019) found that high levels of ERRγ correlate with beta cell maturation, making it a key marker for the functional development of these cells within their protocol[36].

The activation of the Notch pathway has been shown to inhibit glucose-stimulated insulin secretion (GSIS). The primary role of Notch signaling is to maintain the progenitor stage by blocking NGN3, which promotes proliferation after the precursor stage, thus preserving the progenitor state, which avoids premature differentiation into the endocrine cell lines[32]. Also, Notch signaling inhibits GSIS within the in-vitro beta cells by promoting the degradation of MAFA through its detachment from KAT2b. While Notch activation decreases MAFA protein levels, it does not affect mRNA expression. MAFA is known to be crucial for beta cell maturation, and thus, the study suggests that it has a negative effect on beta cell maturity, and GSIS is commonly an indication of it. Additionally, in a comparative analysis, β cell-specific Notch gain-of-function (β-NICD) mice exhibited mild glucose intolerance despite being on the same diet as the control (Cre–) mice, which suggests that insulin is not being secreted properly, leading to the inefficiency of utilizing glucose[31]. This further supports the fact that activated Notch-signaling has a negative impact on proper insulin secretion from the beta cells, and in the context of diabetes, the improper or insufficient secretion of glucose is a common trait. Subsequent deletion of rbpj, a transcriptional effector of the Notch signaling pathway, resulted in the loss of the Notch pathway and reversed the glucose intolerance[31]. Further, it emphasizes the inhibitory role that Notch signaling plays in GSIS if applied within the final maturation stage of the protocol. Braam et al. (2023) utilize the Notch signaling inhibitor, DAPT, at the pancreatic progenitor stage, which regulates the expression of endocrine differentiation. Inhibiting the Notch signaling in that stage allowed for increased expression of NEUROD1, which directs the cells to be committed to being in the endocrine cell stage rather than

any other cell fate[37].

Culture Conditions

Chemical differentiation protocols employ various media conditions to culture cells during their progression from iPSCs to beta cells. Current culture media often contain high glucose levels and amino acids that do not accurately replicate the in vivo environment. This discrepancy can alter cellular metabolism and interfere with endodermal differentiation[38]. A culturing condition that has been tested is hypoxia. Normoxic conditions, with 21% oxygen, differ significantly from the physiological microenvironment of cells, which typically ranges from 1% to 12% oxygen, depending on the organ. Studies have shown that cells cultured in hypoxic conditions exhibit faster growth, reduced DNA damage, and lower stress levels[39]. Pavlacky and Polak (2020)[40] demonstrate that hypoxia can replicate in vivo oxygen availability. Moreover, hypoxic cell cultures with oscillations can accurately reflect in vivo models by simulating periods of high and low oxygen levels, depending on the influence of intermittent hypoxia on the conditions studied[39].

Earlier protocols, such as D'Amour et al. (2005), utilized two-dimensional (2D) culturing methods, where the cells are grown on flat surfaces like Petri dishes. This approach can hinder proper cellular signaling, impacting the efficient differentiation and maturation of beta cells due to a lack of intercellular communication, unlike in vivo conditions. 3D culturing allows cells to grow in scaffolds or matrices, promoting cell interactions which are similar to in vivo. Nair et al. (2019) demonstrated this by aggregating immature beta cells into 3D clusters, which exhibited higher levels of insulin C-peptide which they found were similar to human islets, than those generated through 2D culturing protocols. More recent protocols, such as by Fantuzzi et al. (2022) and Velazco-Cruz et al. (2019), have explored three-dimensional (3D) culturing methods that better mimic the arrangement of beta cells in the pancreas.

In Fantuzzi et al. (2022), researchers investigated two 3D culturing methods for differentiating beta cells: rotating suspension and static microwells. They stained the cultures to quantify glucagon (indicative of alpha cells), C-peptide (indicative of beta cells), and DAPI (which stains DNA). The study found that the suspension technique led to a 40-50% loss of cultured cells due to significant clustering. High levels of DAPI staining suggested cell death, as it might indicate a compromised cell membrane. The loss of cultured cells likely resulted from central necrosis, where core cells were deprived of essential growth factors. In contrast, microwells demonstrated superior outcomes, with 91-100% of experiments successfully avoiding clumping and fewer DAPI-stained cells, indicating less central necrosis. After maturation st7 (mature beta cell; see Fig 2A), microwells produced 5-10% more insulin-producing cells and fewer glucagon-producing cells than the rotating suspension method, suggesting a greater yield of beta-like cells.

Conclusion

There have been many recent advancements in optimizing the differentiation process to culture beta-like cells efficiently and culture cells that most closely resemble the characteristics of in-vivo human beta cells. Changes to the main components of the culturing process, small molecule modulators, and culture conditions have been crucial to the continued progress toward this goal. Key growth factors like Activin A and EGF and small molecule modulators such as Alk5i and ERRγ significantly enhance differentiation. Additionally, hypoxic and 3D culturing conditions mimic in-vivo environments, improving cell proliferation and reducing stress within cells. These advancements can be emphasized by the comparison between the protocol used by Pagliuca et al. (2014) and Fantuzzi et al. (2022).

While Pagliuca et al. (2014) demonstrated a significant step towards producing near-mature beta cells, compared to earlier protocols, by highlighting the cells' ability to perform GSIS, Fantuzzi et al. (2022) further optimized the protocol by emphasizing culture conditions such as 3D culturing, the use of EGF in addition to those used within Pagliuca et al. (2014) which resulted in more maturity and uniformity within the amount of insulin secreted within the GSIS of those cells. Fantuzzi et al. (2022) further made improvements within the two-phase insulin release, which was lacking within Pagliuca et al. (2014), which primarily demonstrated single-phase release, differing from a human beta cell insulin release.

Even though iPSCs have shown potential to be beneficial in disease modeling, certain errors in iPSC formation can negatively affect this ability. For instance, incomplete reprogramming of the somatic cells into iPSC or mutations acquired by the cell during the reprogramming can result in iPSC, which can not correctly function[41]. On the other hand, iPSCs are better at simulating some diseases compared to ESCs due to their ability to retain epigenetic models, which could be used as an advantage to study disease progression from a specific patient[42]. Despite the recent discoveries and advancements within the realm of iPSC to beta cell differentiation, a few limitations must be addressed to optimize these protocols further. An area for improvement could be optimizing the later stages of these protocols. Fantuzzi et al. (2022) highlighted that beta-like cells transplanted into mice exhibited phenotypes more similar to human islet beta cells than their in-vitro counterparts. Identifying and incorporating missing elements from the in-vivo microenvironment into in-vitro cultures could enhance the development of beta-like cells. In vivo, cells surrounding beta cells, such as endothelial cells, play crucial roles by providing necessary nutrients, oxygen, and growth factors. Adding endothelial cells to in-vitro cultures could be beneficial, as they produce essential molecules for beta cell proliferation. A study on rat liver

endothelial cells found that they express Hepatocyte Growth Factor (HGF), which promotes beta-cell proliferation[43].

Using iPSCs to generate beta cells provides the benefit of exploring various therapeutic approaches. One potential therapeutic involves using CRISPR to modify beta-like cells to observe or test treatments. Individuals can be predisposed to diabetes due to inherited or specific genetic mutations. Genome-wide association studies have identified several risk loci affecting type 1 and 2 diabetes. Modifying pathogenic genes in beta cells using CRISPR could allow the patient's own cells to be used in treatment, potentially reducing the need for immunosuppressants after transplantation. CRISPR can be combined with differentiation protocols to create cells with corrected pathogenic variants. For example, Maxwell et al. (2020)[44] conducted an experiment where they harvested cells from a patient with diabetes caused by Wolfram Syndrome. They created fibroblasts from these cells and corrected the pathogenic variants in WFS1, which were thought to inhibit GSIS in beta cells. They differentiated the corrected cells using a cytoskeleton-based protocol. To test the effect of these cells, they implanted gene-corrected, unedited, and cadaveric islets in mice with diabetes induced by streptozotocin injection. They observed that the islets with corrected patient genes showed higher insulin production than islets with unedited patient genes. This instance shows one of the potentials of chemically differentiated cells within personalized therapy for patients with certain types of diabetes.

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