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REVIEW ARTICLE

Insight into (β) Cell Development to Rebuild Bonafide β-Cells: Reprogramming Versus Differentiation of Stem Cells

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ABSTRACT

Background: Pancreatic β-cells for insulin secretion are the main regulators of mammalian metabolic process. Diabetes and hyperglycemia make the patient dependable on exogenous insulin, which is due to the lack of functional β-cells. Oxidative stress also plays an important role in the aggravation of this pathological condition. Recent insights into the development of β-cells along with the pluripotent stem cells discovery have opened new ways to generate β-cells that can help in the screening of various drugs and also in the transplantation therapy since, these pluripotent Embryonic Stem Cells (ESCs) can develop in any type of cell, due to which any defective tissue can be substituted.

Objectives: The objective of this study was to evaluate current strategies which can help to control diabetes mellitus.

Methodology: Methodological approach of this review was based on the comparison of theoretical studies and researches related to diabetes mellitus.

Results: *In vitro* or *in vivo,* replication might be repeated based on proteins or through small molecules. Efforts have been made through which differentiated cells can be converted into β-cells by transcriptional regulators that are the significant players for the development, as well as responsible to identify conditions that cause replication of β-cell both *in vitro* and *in vivo*. The recent strategies can be applied for new β-cells generation and also highlights the future aspects regarding the mechanisms that govern later differentiation stages.

Conclusion: This review provides an update on generating β-cells from different strategies and also brief about the development and function of β-cells that how they could help to control diabetes.

Keywords
Pancreatic β-cells, Oxidative stress, Transplantation, Therapy, Pluripotent stem cells.

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INTRODUCTION

Diabetes mellitus, a metabolic disease caused by the disturbance in regulation of the glucose, consequently induce hyperglycemia, damage tissues and also increase the rate of mortality and morbidity. Pancreatic β-cells secrets peptide hormone-insulin by reacting with high blood glucose that promotes uptake of glucose from blood by different cells in the body e.g., in the liver it undergoes glycogen synthesis (Fig. **1**). Diabetes is further classified into two classes: Type 1 diabetes mellitus is caused from breakdown of insulin developing β-cells, while diabetes

mellitus Type 2 is related to obesity, in which demand of insulin overtakes normal production. Type 2 diabetes develops resistance in peripheral tissues for insulin effects and the excess need often causes de-differentiation, the malfunction and death of β-cells. A report in 2010 by the Center for Disease Control (CDC) figured approximately 25.6 million Americans affected by diabetes mellitus and 300 million people globally, thus worldwide ranking diabetes a major health problem[1]. Approximately 60% of β-cells can be dropped off during Type 2 diabetes and others somehow become dysfunctional. During diabetes, tenacious glucose misregulation can lead to many other complications like retinopathy and related blindness, renal failure, neuropathy and cardiovascular disease[2]. The Centers for Disease Control reported that in Americans, diabetes mellitus is the major cause of renal failure, neuropathy and retinopathy, and because of these complications, costs of healthcare have increased greatly[3]. Such complications could only be controlled by improving glycemic index. To improve glycemic index, many drugs have been introduced in the market, amongst which insulin administration is the common treatment.

Type I diabetes could only be controlled by regular insulin administration, while the treatment of Type 2 diabetes involves oral/injectable therapies that can act upon β-cells. Disease condition may aggravate under the condition of high stress and increased lipid peroxidation, which is verified by number of research studies indicating how the function of pancreatic cells can be altered under the condition of high stress and oxidation[4]. Hence, for the control of the disease condition, number of propositions have been projected that guide how to repair the structure and function of beta cells in our body. Still, none of these cures copes with an exactness of endogenic β-cells, all of these have fallouts which may lead to ketosis and even increase the risk of coma[4]. Such strategies that cause replication of β-cells can raise the available number of β-cells which can insure blood glucose level (Fig. **1**).

Studies have discovered pluripotent embryonic stem cells (ESCs) that can develop in any type of cell, due to which any defective tissue can be substituted. β-cells are good choice for the replacement of cell, as just a single-cell replacement could be done at non-endogenous sites, which is attractive for surgical surgeries. Transplantation is also an effective therapy for diabetics. Further, human β-cells are considered to be beneficial for *in vitro* analyses of metabolism as well as for β-cell functioning. By developing new origins of human β-cells, it would be helpful to develop novel drugs to treat diabetes. This review provides an update on generating β-cells from different strategies and also brief about the development and function of β-cells that how they could help to control diabetes.

Use of Pluripotent Stem Cells to Generate β-Cells

Type 1 diabetic patients lack enough β-cells range and even a lot of them seem to experience none, while Type 2 diabetics also have insufficient mass of β-cells to control glycemic index. Thus, different strategies have been developed for therapeutic replacement by creating new β-cells for the last two decades. The major goal is to identify the pluripotent human embryonic stem cells (hESC) that can generate cells from all embryonic tissues. Discovery of pluripotent stem cell also leads to identify another Induced pluripotent stem cell (iPSC). The most remarkable characteristic of Induced pluripotent stem cells is that, they can give cell types just like embryonic stem cells[5]. Therefore, such cells give new chance *in vitro* to get replacement tissues, which includes autologous cells of individual specific-cells. Following such technique, autologous mouse induced pluripotent stem cells were distinguished into hemopoietic progenitors' cells to treat sickle cell defects, which proved to be effective in anemic mouse model. Similarly, to cure diabetes, such directed differentiated strategies could be made but the only hurdle is to discover such scheme which *in vitro* repeats the growth of functional β-cells (Fig. **2**). During the developmental stage, fertilized embryonic cells select the germ layer that fates to develop β-cells deduce an endodermal layer. Once endodermal specified, developing tissues of adjacent bring on pancreatic progenitor's specification that give rise to entire three types of pancreatic cell: endocrine, acinar and ductal cell (Fig. **3**). Ductal and acinar cells make exocrine pancreatic tissue, while Endocrine pancreatic function is carry out by islets. After endocrine destiny is selected, such progenitors of endocrine must be further specified to five types of islets of endocrine cell: β-cells for insulin production, α-cells for glucagon, δ-cells for somatostatin, ε-cells for ghrelin or polypeptide-producing pancreatic cells. (Fig. **3**). Before a decade, the initial step i.e. the insured endodermic induction had not attained embryonic stem cells. Now *in*

vitro, remarkable advancement has been made in producing β-cells functional of pluripotent stem cells.

Creating Classic Endoderm

Researches in mice, letting insignificant genetic models reported beneath, described such transcriptional factors which are the main governors from pancreatic growth. FoxA2 (Hnf3beta) forkhead box A2 hepatocyte nuclear factor 3-beta and Sox 17(SRY-related HMG-box) are involved in the generation of gut tube and endodermis tissue[6]. The role played by FoxA2 is in part by mediating nucleosome reduction and subsequently activating gene. Nodal, the TGFβ family member have been identified which cause embryonic differentiation within endoderm. Activin, another family member of TGFβ, has the same receptor binding forms like Nodal, easily developed as recombinant protein and can indicate through same downstream pathways. Researchers *in vitro* developed the first effective protocol for definitive endodermic generation via characteristics of activin A from pluripotent stem cells[7].

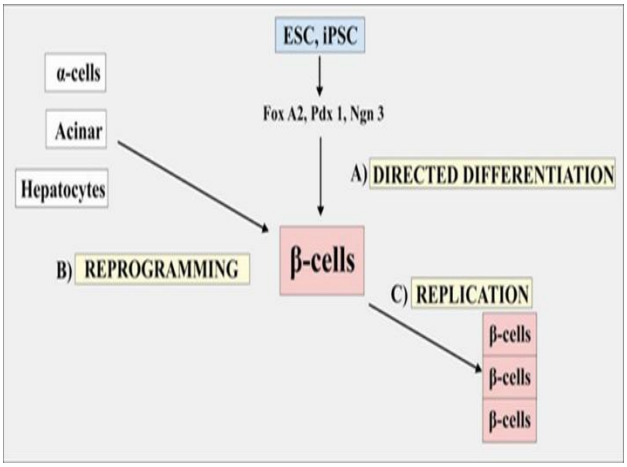

Fig 1. Schemes for new β-cells generation. (A) Pluripotent stem cells can be directed by small molecules and growth factors. At present differentiation of functional β-cell occur only *in vivo*. At every step expression of significant genes is enlisted. (B) Reprogramming of α-cells or acinar cells *in vivo,* used to give β-cells while *in vitro* reprogramming of other cells like fibroblasts, hepatocytes and neurons persist to be accomplished. (C) Making already β-cells able to replicate is the main strategy to generate new β-cells. *In vitro* or *in vivo,* replication might be repeated based on proteins or through small molecules.

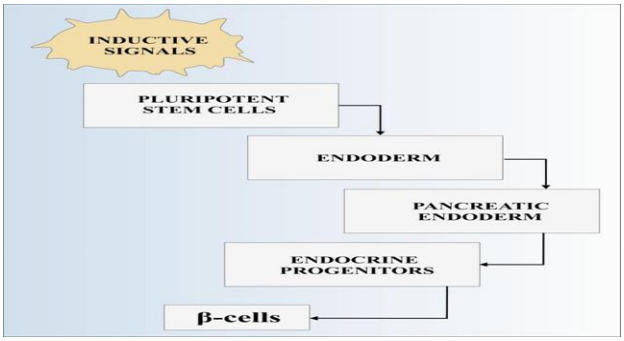

Fig 2. General development of β-cells. Pancreatic β-cells growth and related transcription factors which are needed for lineage specificity. β-cells, α-cells, δ-cells, ε-cells and pp-cells release insulin, glucagon, somatostatin, pancreatic polypeptide and ghrelin, respectively.

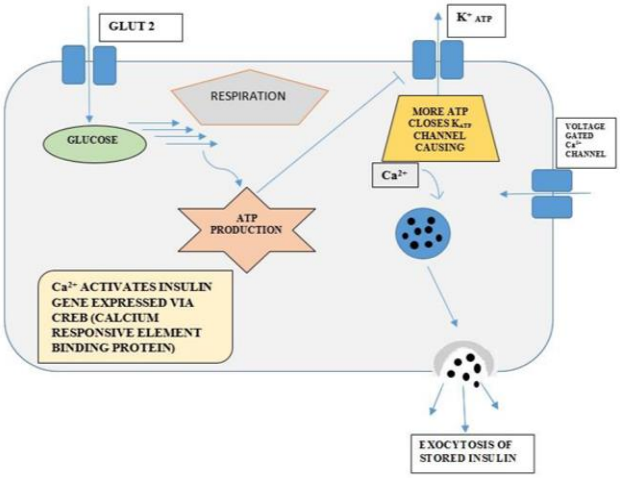

Fig 3. Mechanism of insulin secretion into pancreatic β-Cells. Inside the β-cells production of insulin, regardless of blood glucose level is somehow constant. Its sortation takes place inside vacuoles, through exocytosis, that is mainly activated via food having glucose.

Asserting Function and Identity of β-Cell during Culture *In Vitro*

To explain cultures of ESC-derived deficiency of β-cells, a third hypothesis is that although existent protocols are can generate β-cells still *in vitro*, such conditions of cultures are still lacking through which these cells could be maintained or new β-cells can be trained such that they appropriately react to glucose. *In vitro*, generally to keep human β-cells functional and alive seems difficult. It is reported that human islets culture after 48-72hrs, results in one-half of the islet cells lost and *in vitro* glucose stimulated insulin secretion (GSIS) reduced dramatically[8]. According to this

information it has been suggested that by a strategy of directed differentiation, generation of β-cells can occur but their function and survival rate is not very considerable utilizing current protocols. Yet, several reports suggest that *in vitro* β-cells could survive for a few weeks by using such certain cultures in which cells after transplantation, can maintain their function.

The environment to maintain cells end function *in vitro* should be similar like *in vivo*. Once, cultures of human islets are made functional and differentiated, the components of islet niche are present which includes side by side mesenchymal and endothelial cells and extracellular matrix. *In vitro*, by the addition of proteins of extracellular matrix in ESC-insulin (Embryonic Stem Cells), positive cells function and end differentiation might ameliorate. Extracellular matrix, neuronal, mesenchymal, endothelial and exocrine cells surround the human islets, among which many play its role for function and identity of β-cell, for example, expression of Pdx1 is maintained by endothelial cells[9] and causes expression of Ptf1a and endothelial cells thus, get attracted by growing islets through VEGFA secretion. Within hESC, definitive endoderm replication can be caused by mesenchymal cell lines of specific organs[10]. Eventually, it has been noticed that the function and differentiation of authentic β-cells are in three-dimensional way, whereas many research attempts have been made in two-dimensional culture. According to these observations, the generation of functional β-cell can be improved by making changes in culture conditions (Fig. **4**).

Types of Reprogramming into β-Cells

Another substitutive strategy of β-cells differentiation from pluripotent stem cells is the reprogramming from marked cells to β-cells. Due to such direct reprogramming, it has been made possible to generate hepatocytes, iPSCs induced pluripotent stem cells, neurons and cardiomyocytes[11]. Remarkably, in each case just 2-4 genes are needed to be overexpressed. For the last few years, reprogramming of other cells into β-cells has been proved successful.

Re-Programming of Acinar into β-Cells

In vivo, this approach of reprogramming involves acinar cells of a mouse directly into β-cells through a viral expression of a specific gene. This proved that initially nine transcription factors into β-cells were expressed, among which Mafa, Pdx1 (GLUT and duodenal homeobox 1) and Ngn3 (Neurogenin-3) constituted enough which formerly converted into acinar cells and later these cells can be reprogrammed into β-cells. *In vivo*, through lineage tracing, it has been proved that Cpa1 could be converted into post-transductional insulin expressing cells. Significantly, central markers of functioning β-cell which includes Glut2 (Glucose transporter 2), Slc2a2 (solute carrier family 2 members 2), glucokinase and Nkx6.1 can be co-expressed by insulin-expressing cells, but main regulators of acinar i.e. Ptf1a/ amylase and other hormones are not expressed for longer.

In the end, in diabetic mice, these stimulated cells were enough to control the glycemic index, the major function of β-cells. However, universally for β-cells generation, these cells are not enough, as they are not enough for reprogramming of fibroblasts or skeletal muscle into β-cells. Though *in vivo*, reprogramming of acinar cells had been done *in vitro* none of the cells of human or mouse could be reprogrammed completely[12]. Moreover, acinar cells which had been reprogrammed cannot combine in islets may be due to the deficiency of other types of reprogrammed islet cells like α-cells, therefore, just β-cells are generated from these factors. Almost significantly in the circumstance of a potential cure, this accomplishment will require repetition free of viruses *in vivo* or maybe *in vitro* for the safer side, for subsequent transplantation of cell. The second most significant goal is to attain from human cells.

Reprogramming from α-Cells to β-Cells

Adult mouse α-cells have been used to elaborate adult cell reprogramming to β-cells. Mansouri and colleagues found ectopic expression of Pax4 (Paired box 4) responsible for conversion of α-cells into β-cells *in vivo*[13]. On the other hand, loss of Pax4 results in a decreased number of β-cells and increased α-cells. This shows the developmental pathway of these two cell types and their same phenotypic expression.

Similarly, removal of near-complete β-cells may induce reprogramming into α-cells. Herrera and co-researchers, using diphtheria toxin receptor system, introduced transgenic mouse modelling which allowed tracing of α-cell lineage and almost complete ablation of β-cells[14].

New β-cells were regenerated from prior α-cells after complete destruction of β-cells. These transgenically marked α-cells co-express the markers of β-cells i.e., Nkx6.1 and Pdx1, thus producing insulin. Interconversion of these two cells is probably due to the same physiology and development history which is possible in extreme situations that completely lacks signalling of local insulin. Studies disclosed that α-cells persistently entertain bivalent chromatin signatures at genes just like β-cells, like Pdx1 and MafA (Musculoaponeurotic Fibrosarcoma Oncogene Homolog A)[15]. Chromatin bivalent signature consists of active and passive marks of histone which describes that α-cells might regenerate into β-cells due to active β-cells particular genes. Expression of insulin and Pdx1 in glucagon-positive cells is a consequence of islets treatment along with the inhibitor of histone methyltransferase, which depicts the ability of partial reprogramming in these cells. It is not observed till now whether complete α-cell to β-cell trans-differentiation is possible, however, under controlled immune regulatory conditions in serious Type I diabetes mellitus patients, it may be possible to minimize β-cell destruction and not α cell trans-differentiation. It might be assumed that in the absence of β-cells, transplantation of hESC-derived α-cells may induce trans-differentiation of these α-cells into β-cells *in vivo*. To strengthen this hypothesis, *in vitro* α-cell trans-differentiation is either via Pax4 overexpression or through some modulations of insulin/glucagon levels in the niche may be supportive enough. Genetic manipulation ability of human pluripotent stem cell enables a researcher to sketch out the progeny of *in vitro* derived α-cells using glucagon promoter and observing differentiation in culture or after transplantation into mice[16].

Reprogramming of Other Types of Cells

A specific injury to the pancreas i.e., Partial Duct Ligation (PDL) is discussed to increase islet cells by trans-differentiation of adjacent tissues[17]. During this injury, pancreatic ductal cells increase in number rapidly but at the same time acinar tissue is destroyed. Based on Xu and co-researchers proposed that PDL can trigger Ngn3 (Neurogenin 3) re-expression as a result of which progenitors differentiate into new β-cells[18]. Molecular inducers for this conversion are still unknown and its occurrence in humans or *in vitro* is also under discussion. The study revealed that ductal cells were responsible to trigger expression of Ngn3 trans-differentiate in endocrine cells, but ductal-specific lineage retracing elaborated that ductal cells of Sox9-positive (Sex determining region Y-box 9) are not responsible for the production of β-cells after partial duct ligation or β-cell ablation[19]. In the same way, many researchers revealed that a number of entire β-cell might not alter after PDL and increase in β-cell may be a consequence of methodological estimation. While a recent study indicated that after PDL, Ptfla-positive acinar cells can give rise to Ngn3-positive progenitors which eventually distinguish into novel endocrine cells even at a very lower rate[20]. Ptfla-positive acinar cells produced insulin-expressing cells (makers of mature β-cell) Pdx1, Nkx6.1, MafA. These cells resemble with β-cells deduced of virally programmed acinar cells. These lineage-tracing studies after PDL revealed that postnatally, β-cell neogenesis performs the limited role to increase β-cell mass. In this regard, the researches so far indicate contradictory views and a limited capacity of pancreas to regenerate and capacity of β-cell neogenesis to increase β-cell mass[21]. For the treatment of diabetes, the function of reprogramming still needs discussion and research. Terminally marked cells can be converted to other types of cells via different over-expression strategies of reprogramming. These strategies should be examined *in vitro* as well as *in vivo,* for better validation. This would be the next step, rather the simple detection of insulin expressing cells and reprogramming factors like Pdx1 can trigger the insulin promoter directly. Colonial β-cell genes' induction (Glut2 and Nkx6.1) is more significant for cell fate and reprogramming than the insulin expression only. Comparison of genomic profile of reprogrammed β-cell and endogenous β-cell will show how reprogrammed cell is closed with the already existing β-cell. For further evidence towards this comparison and similarity of both cells, *in vitro* GSIS attempts, transplantation and host blood sugar level regulation will prove more than effective. Another objective is to identify the reprogramming of approachable tissues, direct reprogramming of ESCs/fibroblasts and more significantly human tissues. Eventually, the main target for reprogramming should be misregulated insulin-expressing cells produced from pluripotent stem cells.

Regeneration of β-Cells Caused Through Replication of β -cells

Cell neogenesis might be accomplished by reprogramming via pluripotent stem cells as well as by induced replication of endogenous β-cells (Fig.**1c**), while tissues are regenerated by differentiation of tissue-specific stem cells. Replication of existing β-cells can regenerate new pancreatic β-cells[22]. Endogenous β-cells are the autologous source of β-cell regeneration, hence, decreasing the responsibility of existing β-cells which overwork in Type 2 diabetes. Furthermore, a study indicates that almost 16% of patients of Type 1 diabetes retain some β-cells and their function can be estimated by detectable C-peptide levels[23]. Proinsulin processing secretes C-peptides or other peptides within equimolar insulin ratio. Presence from functional β-cells within patients indicates their potential to regenerate β-cells from therapies inducing replication of the residual cells but these therapies should be supportive enough to combat autoimmune attack. A specific risk associated with this scheme is the accidental advancement from tumorigenesis. This risk is high when proliferation starts from acinar or ductal tissues along with the β-cells. This risk can be minimized by selecting replication-inducing agent of choice having a higher specificity towards β-cells instead of other types of cell.

Rates of Replication are Higher in Young β-Cells

Human aging decreases the replication process from 3% of fetal β-cells to 0.5% with the age of 6 months and even lower thereafter[24].In another research, β-cell replication in young mice (5 weeks old) was 2.5% while old mice had only 0.2% replication rate. Replication of β-cell in Diphtheria toxin based β-cell ablation model is 7.5% in young mice while 1% in old. This data suggested that the replication decreases with the aging process. This also suggests that intrinsically, the capacity to replicate is also present in older ages but the β-cell replication rate is greater among youth. The variability of the replication rate is because of systemic aging factor. β-cell replication accelerates during pregnancy probably due to activities of prolactin and placental lactogen. However, the mass of β-cell is lower among humans as compared with rodents[25]. Prolactin acts by repressing transcriptional regulatory protein, menin, which can prevent pregnancy associated replication. On the other hand, pregnancy influences the secretion of insulin despite a number of β-cells.

Testing of Smaller Molecule Factors Through Replication Regulates

When Glpr1 agonist (Glucagon-like peptide 1 receptor) was treated with exendin-4, it resulted in three times increase in BrdU-positive replicating β-cells[26]. Exendin-4 is more useful than glucagon like peptide 1 because of longer half-life. The replication inducing effects of exendin-4 can be determined in young human islet (<22 years) transplantation in mice rather than the old islets (>35 years)[27]. Higher turnout testing from reversible immortalized β-cells/ rodent islets indicates molecular regulators for β-cell replication i.e., thiophene-pyrimidines, adenosine kinase inhibitors, phorbol esters and dihydropyridine derivatives. Glpr1 agonists and glucose have synergistic effect for β-cell replication. But their inductive effect on humans is still unknown. Recently, Schultz and co-researchers traced an important small molecule WS6, which accelerated β-cell replication in intact islets more than ten times and in dispersed islets up to six times[28].

Identification of Replication Mechanism by Utilizing LIRKO

Liver-Specific Insulin Receptor Knockout (LIRKO) mice were produced to explore the function of insulin signaling within hepatocytes in the development of diabetes. Mutation of insulin receptors resulted in glucose intolerance, insulin resistance as well as up to six times increase in islet mass. This study suggested that insulin receptor blockage with peptide antagonist or small molecule sum up the effect of β-cell replication induction. Antagonist insulin receptor S961 treatment resulted in hyperinsulinemia in mice. Another study of LIRKO model suggested that it doesn't affect α-cells or other non-pancreatic cells except β-cells[29]. This study also illustrates that replication may be induced when a parabiotic partner is brought together on LIRKO (Liver Insulin Receptor Knockout) mouse. This suggests that general component can function independently of blood glucose levels. In addition, to summarize replication induction in isolated mice islets *in vitro*, serum from LIRKO-derived or LIRKO liver explants system was quite enough but responsible factors are not traced yet.

Function of Glucose and Resistance of Insulin During Replication of β-Cell

Glucose itself is responsible for induction of β-cell replication in humans as well as rodents. But in diabetes mellitus case such replication is not enough to meet the requirement or autoimmune assault. Glucokinase mutation (V91L) increases its affinity for glucose resulting in greater islet mass and β-cell replication. Insulin resistance created due to high-fat food induces β-cell replication. In 20 weeks, under such conditions, β-cells replication is more than doubled but this requires glucokinase, because glucokinase, deficiency decreases the induction of β-cells replication. On the other hand, glucokinase inducers can increase the induction of β-cells replication up to two folds in both young and old mice[30]. Induced β-cell replication may also be a beneficial side effect of antidiabetic therapeutics dependent upon glucokinase activation. Insulin resistance can also stimulate organ specific transcriptional changes to trace out the factors which stimulate replication of β-cell. Hepatocyte analytical transcriptional alterations of S961suggests upregulation from betatrophin hormone. Betatrophin induction increases β-cells replication more than ten folds irrespective of insulin antagonism. So, this newly discovered hormone may help to suggest a new therapeutic approach towards producing more β-cells.

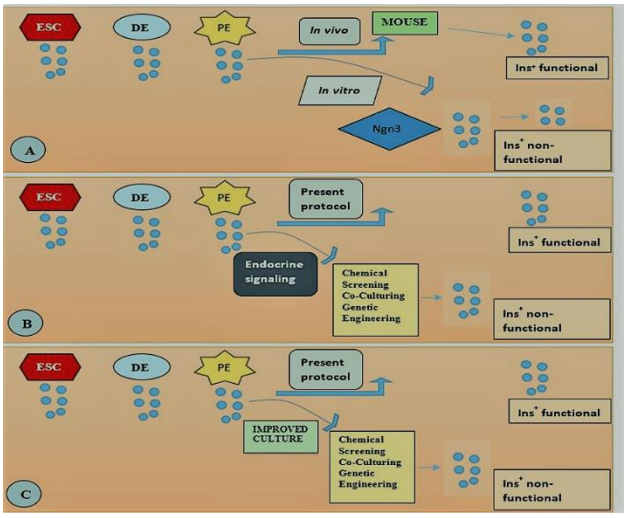

Fig 4. Distinguishing how to ameliorate β-Cells differentiation. (A) *In vivo,* this lineage tracing of ESC reveals which cells are capable of generating functional β-cells while in vitro cells might turn on the expression of genes but would not be functional. (B) *In vitro*, chemical screening, co-culture or genetic engineering techniques may be helpful to trigger the endodermic distinction into functional cells of endocrine. (C) *In vitro*, recognition of new improved culture can increase the chance to generate β-cells.

CONCLUSION

In conclusion, new advancement has been made in developing new β-cells since the last decade. However, at the same time challenges other than those which are discussed above persist. Particularly, this review is limited to evaluate β-cell, yet the knowledge covering the problem of an immune system will be significant to treat Type 1 diabetes whether via new β-cells transplantation within an immuno-protective capsule or through general an immuno-modulation. In case of transplanting new β-cells into Type 2 diabetic patients as to improve the glycemic index, the consequence of immune system has to be considered. In this respect, strategy to consider the issue of immune is to encapsulate new β-cells in an immuno-protective device which allows diffusion of nutrients merely inhibiting infiltration of immune cell. One of the successful devices has utilized human islets transplantation and also hESC progenitors into mice. The substitute is to develop such protocols for reprogramming or differentiation of iPSCs in β-cells, especially iPSCs generated through non-viral methods. Formerly, studies had proposed that immune assault occurs in syngeneic mice by transplanting undifferentiated iPSCs. Still, there is no involvement of transplanting undifferentiated pluripotent stem cells in patient-particular to iPSC therapy.

Recently, other studies also proved that iPSCs differentiated cells on transplantation generate no immune response and that previous studies results may be utilizing iPSCs derived retrovirally[31]. Therefore, in the future, iPSC-gained β-cells can have a beneficial role in therapies by developing such scientific strategies which can defeat heterogeneity of differentiated tendencies in a single cell line. Utilization of sources of new β-cells needs to give such homogenous population of a cell which have a deficiency of multipotent cells so that tumors or cysts could not develop. At present, enormous progress has been made in directed differentiation of early stage which is 99% but 100% results could be achieved for later stages once cues of proper development are exposed. Also there is a need

to generate these cells at large scale and recently this first effort had been made successful[32]. In conclusion, enormous progress has been made to understand how through new methods β-cells grow, multiply and function during normal development. Still, the challenge is *in vitro,* that how to rebuild bonafide β-cell, either via reprogramming or differentiation of stem cell.

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LIST OF ABBREVIATIONS

CDC	Center For Disease Control
ESCs	Embryonic Stem Cells
GSIS	Glucose Stimulated Insulin Secretion
LIRKO	Liver-Specific Insulin Receptor Knockout
Ngn3	Neurogenin-3
Pdx1	Pancreatic and Duodenal Homeobox 1

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