GLOBAL ACADEMIC RESEARCH INSTITUTE

COLOMBO, SRI LANKA

GARI International Journal of Multidisciplinary Research

ISSN 2659-2193

Volume: 05 | Issue: 05

On 31st December 2019

http://www.research.lk

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GARI Publisher | NCD | Volume: 05 | Issue: 05

Article ID: IN/GARI/ICHM/2019/211 | Pages: 70-78 (08)

ISSN 2424-6492 | Edit: GARI Editorial Team

Received: 27.11.2019 | Publish: 31.12.2019

PANCREATIC β CELL REGENERATION FROM HUMAN EMBRYONIC STEM CELLS AS A POSSIBLE THERAPY FOR TYPE 1 DIABETES

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ABSTRACT

Type 1 diabetes (T1D) is an autoimmune disorder characterized by the destruction of functional insulin-producing pancreatic β-cells caused by autoreactive T cells. Two possible approaches for replenishing the β-cells are replacement by transplanting cadaveric islets or β cells derived from induced pluripotent stem cells (iPSC) or human embryonic stem cells (hESC) and induction of endogenous regeneration. As of today, the use of pluripotent stem cells (PSCs), including embryonic stem cells (ESCs) and induced PCSs (iPSCs) is one of the most promising therapeutic approaches. Production of glucose-sensitive, insulin-secreting beta cells derived from pluripotent hESC is an ideal cure for the treatment of diabetes. However, studies suggest the need to improve induction approaches as the production of insulin of these cells is lower than that of the endogenous pancreatic β-cells. The current review focuses on an overview of the advances in the generation of hESC-derived pancreatic β cells, generation of insulin-secreting islet-like clusters from hESCs in vitro and how the in vivo environment responds to glucose by the secretion of human C peptide and insulin when transplanted into animal models and the limitations and challenges of this therapy for the successful treatment of diabetes. Thereby, emphasizing the fact that further maturation of differentiated β-cells will be able to generate insulin-secreting cells for transplantation into patients with T1D as a potential therapy. Currently, the first phase 1/2 clinical trials with ESC-derived pancreatic progenitor cells are ongoing.

Keywords: Type 1 diabetes, pancreatic β cells, human embryonic stem cells

INTRODUCTION

Diabetes mellitus is characterized by persistent hyperglycemia due to an impaired ability of the body to secrete or respond to insulin, or combination of both (Kharroubi and Darwish 2015). Diabetes mellitus can be classified as type 1 diabetes (T1D), type 2 diabetes (T2D), gestational diabetes (GDM) and other types by etiology and clinical presentation. Type 1 diabetes (juvenile diabetes or insulin-dependent diabetes) is an autoimmune disorder which is more common in children and teenagers (Goyal and Jialal, 2018). A study by Evans et al. in 2016 shows that among 415 million diabetic patients 10% are T1D patients (Figure 1). Pancreas plays the role in the energy consumption and metabolism. Less than 5% of total pancreatic mass represent the endocrine islets which synthesizes hormones (Zohu and Melton, 2018).

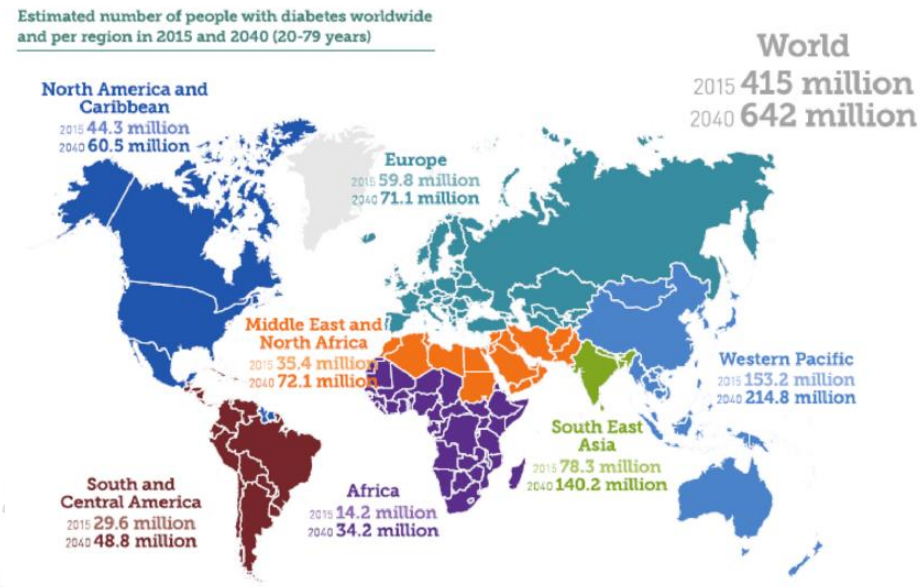


Figure 1. Worldwide prevalence of diabetes; 2015 and 2040 (Evans et al., 2016).

The autoimmune destruction of insulin producing β cells which is present in the islets of Langerhans will lead to hyperglycemic conditions. Continuous destruction or degeneration of beta cells causes T1D. Diagnosis of diabetes is done according to the criteria given in Table 1 below. Prolonged T1D disease may cause blindness, kidney failures, heart attacks, stroke and premature dying. Diabetes can be treated and the negative consequences can be avoided (Cito et al., 2018).

Table 1: Diagnosing Diabetes (adapted) (Kahanovitz, Sluss and Russell, 2018)

Test	Results	Interpretation
HbA1c	≥ 6.5%	Diabetes
	5.7-6.4%	Impaired glucose tolerance
	≤ 5.7%	Normal
Fasting Plasma Glucose	≥ 126 mg/dL (7.0 mmol/L)	Diabetes
	100–125 mg/dL	Impaired glucose tolerance
	≤100 mg/dL	Normal
OGTT	≥ 200 mg/dL (11.1 mmol/L)	Diabetes
Random plasma glucose	≥ 200 mg/dL (11.1 mmol/L)	Diabetes
	140–199 mg/dL	Impaired glucose tolerance1
	≤ 140 mg/dL	Normal

There are number of approaches performed on T1D patients as shown in Table 2. The most significant therapeutic event in the history of type 1 diabetes was the discovery of exogenous insulin (Atkinson, Eisenbarth and Michels, 2014). Along with its therapeutical advancements it has been a major convenient therapy till date in most of the developing countries (Iqbal, Novodvorsky and Heller, 2018).

Table 2: Cons of β cell therapies (adapted) (Rodeman and Hatipoglu, 2018).

Year	Strategy	Cons
1922	Exogenous insulin	• Short-term durability of the glucose sensor (about 1 week) • Not a permanent treatment effect • Long term administration
1966	Pancreas transplantation	• Donor shortage • Life-long immunosuppressive treatment • Open surgical procedure
1977	Islet transplantation	• Need to 2 to 4 donors per recipient • Life-long immunosuppressive treatment • Improvements are needed in harvesting islet cells and increased survival of transplanted donor cells • Alloimmunity

Despite the progress of these therapies, due to the above-mentioned problems, scientists have focused on stem cell research which may pave way into a realistic treatment for diabetes in the near future. Cells which have the potential for unlimited or prolonged self-renewal and to generate various other mature cell types are referred to as stem cells (Chagastelles and Nardi, 2011).

This literature review will focus on the in vitro pancreatic regeneration of β cells from ESCs which is a current ongoing clinical approach for T1D. Embryonic cells, first isolated from human embryos in 1998 are generally preferred as they are pluripotent and are able to differentiate into 200 cells representing all three germ layers; they are immortal in culture and will not senesce after several passages; and they maintain a typical chromosomal composition while adult stem cells have limited potency and differentiate into cell types of their own origin (Mahla, 2016; Chagastelles and Nardi, 2011).In this therapy, autologous embryonic cells are not used but cultured embryonic stem cells are prompted to differentiate in vitro, and thereby, the differentiated cells are implanted into patients. Currently, human embryonic stem cells, (hESCs) are the only stem cell population which are able to proliferate at a rate of >250 population doublings per year. These cells are capable of efficiently and rapidly differentiating to produce cells of all somatic lines by a series of defined developmental transitions (Kroon et al., 2008).

Stem cell therapy to reverse diabetes

Human embryonic stem cells (hESCs) are derived from human embryos that are a week old referred to as blastocysts. The lack of islet donors required for the treatment of T1D could be compensated by the development of hESC-derived insulin producing β-cells. Recent approaches in stem cell research have been successful in generating in vitro hESC-derived β cells. These cells have shown promising results when transplanted into animal models. But however, hESCs derived insulin-producing cells show deficiency in many functional characteristics compared with adult human pancreatic β cells. Below mentioned are a few recent clinical trials which have provided evidence to aid in the reversal of diabetes in animal models.

β- cell replacement in mice using hESC lines derived by somatic cell nuclear transfer

The process of nuclear transfer involves the removal of DNA from an immature egg cell and enucleating the donor oocyte with the genetic material derived from a body cell of the patient who is to undergo the embryonic stem cell transplant. Thus, embryonic stem cells are created by the removal of the inner cell mass of the pseudo fertilized egg once it reaches the blastocyst stage (Fortier, 2005). Nuclear transfer-derived human embryonic stem cells have recently been shown to produce insulin generating β-cells. Sui et al. in 2018 examined the ability of NT-ESs derived from a T1D patient to differentiate into functional β-cells and provide a source of autologous islets for replacement in diabetic mice. The differentiation capacity of 1018-NT-ES-derived β-cells was assessed by the result in the expression of endodermal genes as shown in Table 3.

Table 3. Gene expression of differentiated β-cells at each maturation stage (Sui *et al.*, 2018).

β- cell stage	β-cell marker	Percentage of gene expression
Pancreatic progenitor stage	SOX17	~95%
	FOXA2	~95%
	PDX1-	~97%
	Co-expression of PDX1 and NKX6.1	~80%
β-cell stage (27 days of differentiation)	PDX1	~80%
	C-peptide	~55%

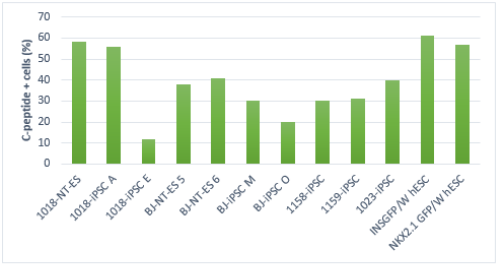
Moreover, various cell lines as shown in **Table 4**, were used to compare the differentiation potential of β-cells into C-peptide–positive cells.

Table 4: Information on hESC lines and iPSC lines derived from type 1 diabetic patients and healthy subjects (Sui *et al.*, 2018)

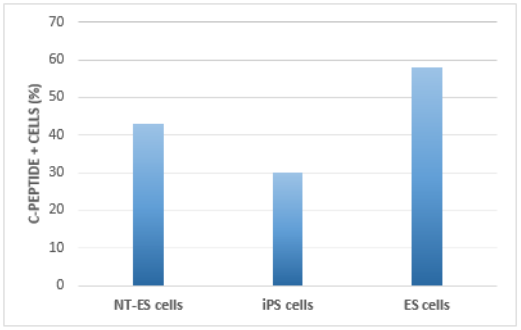
ID	Diagnosis	Stem cell line ID
1018	T1D	1018-NT-ES 1018-iPSC A 1018-iPSC E
BJ	Healthy control	BJ-NT-ES 5 BJ-NT-ES 6 BJ-iPSC M BJ-iPSC O
1158	T1D	1158-iPSC
1159	Healthy control	1159-iPSC
1023	Healthy control	1023-iPSC
$INS^{GFP/W}$ hESC	Not applicable	$INS^{GFP/W}$ hESC
NKX2.1 $^{GFP/W}$ hESC	Not applicable	NKX2.1 $^{GFP/W}$ hESC

It was noticed that the number of C–peptide positive cells obtained from isogenic 1018-NT-ES was significantly higher than 1018-iPSC E cell line. The number of C-peptide cells were also reduced in the BJ- human induced

pluripotent stem cell line- O compared with that of the isogenic BJ-nuclear transfer embryonic stem cell line. On average, iPSC cell lines showed a reduced C-peptide differential potential (Graph 1 and 2).



Graph 1: Comparison of the derivation of C-peptide positive cells from various cell lines (adapted) (Sui *et al.*, 2018).



Graph 2: Overall comparison of NT- ES, iPSC and ES cell lines (adapted) (Sui *et al.*, 2018).

Upon transplantation, 1018-NT-β-cells were able to cause the reversal of diabetes in animal models by secreting insulin during high glucose concentrations in blood, and decrease insulin secretion during low levels of blood glucose. These results provide evidence of how suitable the NT-ES derived β-cells are for the substitution of islets in patients with T1D. Further studies are required to determine the molecular basis for the differences in efficiency of the in vitro differentiated cells compared with the human pancreatic β-cells.

Influence of the in vivo environment upon transplantation of differentiated β-cells.

The lack of islet donors required for the treatment of diabetes could be compensated by the generation of hESC-derived β-cells to restore normoglycemia in diabetic patients. No complete physiologically active β-cells could be generated in vitro although various protocols have been established for the derivation of pancreatic progenitors from hESCs. Sui et al., (2013) conducted a study to examine the in vivo growth of these cells obtained from hESCs following transplantation in the fat pad or subcutaneous site. Twenty-one (21) mice in total were implanted with PDX1-(pancreatic and duodenal homeobox 1) positive pancreatic endoderm (PPP) cells either into the epididymal fat pad or the dorsal subcutaneous space. It was observed that 2 weeks after implantation only few PDX1+ cells remained in the fat pad site. Subcutaneous grafts in 6 weeks' time continued to express PDX1 at all analysed time points and after a period of 6 weeks exhibited co-expression of PDX1 and homeobox protein NKX6.1. Furthermore, between 6 and 12-weeks post transplantation they generated NGN3+ cells as well as some C-peptide positive cells. In addition, 6 weeks post implantation cartilage tissue developed in the fat pad from contaminating MSCs present in the graft, but not in the subcutaneous space.

According to this study, it tends to be presumed that the in vivo microenvironment contributes in the further differentiation of the implanted PDX1+ β cells. As of now, it is not yet known as to why the transplanted hESC-derived pancreatic β-cells responded differently at the two locations. Probably, it is because the subcutaneous site provides the required factors for the survival and proliferation of the cells that the fat pad does not. A similar study was

conducted by Matveyenko et al. in 2010 where hESC-derived PDX1+ / NGN3+ (Neurogenin-3) cells was transplanted into the epididymal fat pad in a total of 15 nude rats. However, after a period of 20 weeks post- transplantation, a small amount of differentiated β cells were detected in only half of the grafts implanted. The failure of the development of the pancreatic endoderm into remarkable physiologically active glucose-sensitive insulin secreting cells when implanted into the fat pad in the nude rats as implanted previously in mice can be due to various possible causes. It is unlikely to be due to technical issues of implantation or insufficient cells implanted. It is conceivable that the nude rat model is a less accommodating host than the nude mouse model post-transplantation of the PDX1+ pancreatic endoderm cells. Additionally, Eshpeter et al., (2008) and Phillips et al., (2008) could not identify any insulin secreting β-cells until 6 weeks post transplantation, and the transplanted diabetic animals did not show any significant decrease in the levels of blood glucose. It was assumed that different results could be obtained if the transplantation time was prolonged in order to allow further differentiation of the NGN3+ progenitors into mature insulin producing β cells as Sui et al., (2013) detected insulin producing cells 12 weeks post transplantation. Analysing the data from the above-mentioned studies supports the beneficial effect of an in vivo environment. However, unfortunately the impact of such as environment is not known in precise.

hESC-derived β cells restores blood glucose levels through intra-spleen migration.

Extensive investigation of the implantation of insulin-producing cells derived from embryonic stem cells (ESC) has been taken place in search of a cure for T1D. However, the mechanism of the transplanted cells in vivo remains uncertain and needs further investigation in diabetic animal models. There have been significant shortcomings such as scarcity of animal models at scheduled timings, lack of studies on a longitudinal basis in the same organism and constrained usage of them in clinical studies in obtaining facts regarding the in vivo behaviour of transplanted pancreatic like β-cells in diabetic mice via histological means. Thus, the urgent need for a non-invasive approach for assessing cell distribution and migration for both human and animal trials in stem cell-based studies arose over time. Ren et al., (2014) investigated the site and movement of insulin-producing cells labelled with super-paramagnetic iron oxide (SPIO) through dynamic MRI (magnetic resonance imaging) as it is an appropriate tool to characterize anatomic information in a non-invasive way. After transplantation of SPIO labelled insulin generating cells into the renal sub-capsular area of diabetic mice, hypo-intense signals appeared in the spleen after one week of transplantation and turned out to be progressively evident by the fourth week and remained as such over the entire study period while the MRI signal intensity under kidney sub-capsules declined to nearly 60% after 6 weeks of cell delivery (Figure 2).

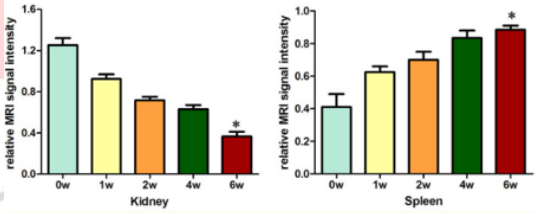


Figure 2: Relative intensities of MRI signals in kidney and spleen over a 6-week period (Ren *et al.*, 2014).

Furthermore, this study compared the glucose levels in blood and the rate of survival of cells when transplanted into renal sub-capsules to those of cells transplanted into the spleen. Each transplantation group consisting of a

sample number of 10 diabetic mice. Results demonstrated that blood glucose control was reached in spleen by the fourth day post- transplantation, ahead of the transplantation in the kidney sub-capsules (Figure 3). But, no statistical significance (62.5%) was detected in the rate of survival of the cells in both the spleen and kidney transplantation group by the end of the study.

As demonstrated by the results, it is not through a direct pathway that the migration of C-peptide positive cells takes place from the kidney into spleen but via the circulation of blood. However, the molecular mechanisms involved in the migration of the grafted cell remains doubtful. The reason for cell migration into the spleen is assumed to be the good vascular supply and the oxygen-rich microenvironment for the islets which the kidney sub-capsules unfortunately lacks. Usage of MRI allows repeated observations in the same living organism, which would expand our comprehension of in vivo stem cell behaviour post-transplantation and could help in the advancement of therapies for the treatment of T1D diabetes. But results have been varied in a study conducted by Bruin et al., (2013) where he transplanted hESCs derived pancreatic progenitor cells under the kidney capsules and found it out to be a suitable location for the transplantation of cells in rodent models. However, a major limitation of that study was the cell formation resembling bone and cartilage. Taken together, these studies confirm that under a range of in vivo conditions pancreatic progenitor cells derived from hESC are capable of treating T1D.

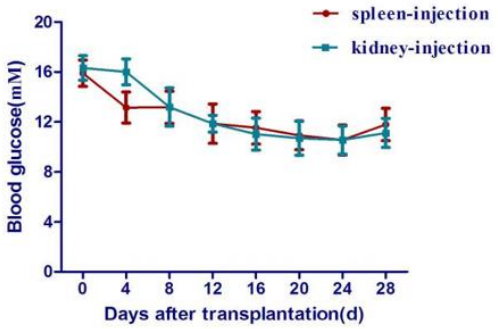


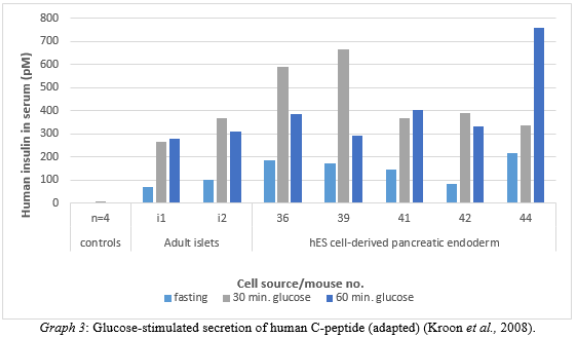
Figure 3: Blood glucose levels in the groups transplanted into kidney sub-capsules (n=10) (blue lines) or into spleen (n=10) (red lines) (adapted) (Ren *et al.*, 2014).

Generation of glucose-responsive insulin-secreting β-cells by hESCs derived pancreatic endoderm

Production of functional insulin secreting β-cells that respond to glucose would aid in the advancement of a cell-based therapy for T1D. Jiang et al., (2007), developed a 36 day protocol where hESCs were differentiated into islet like clusters with characteristics like those of pancreatic β cells in the presence of signalling molecules and specific growth factors in vitro. It was observed that the hESC-derived islet like clusters exhibited multiple β-cell specific genes as mentioned in Table 4. By day 36, Pdx1+ cell clusters differentiated further and in response to the stimulation of glucose, cells secreted pancreatic hormones such as insulin, glucagon and somatostatin. To add to this study, Kroon et al. in 2008 showed that pancreatic endoderm derived from human embryonic stem (hES) cells efficiently generate endocrine cells that respond to glucose upon transplantation into in mice. Upon glucose administration in implanted mice, C-peptide and human insulin were detected at similar levels to those of mice implanted with approximately three thousand adult human pancreatic β cells. Moreover, the insulin-expressing cells post engraftment exhibited many characteristics of physiologically active β-cells, including

expression of mature endocrine secretory granules, transcription factors belonging to the beta- islets and appropriate processing of proinsulin. The differentiated pancreatic β cell clusters were distinguished by the co-expression of FOXA2, PDX1 and HNF6; NKX6-1 was also expressed by the majority. The differentiated β- cells were implanted into both epididymal fat pads in 105 male immunodeficient mice.

Thirty-sixty days post transplantation the team assessed if the human embryonic stem cell–derived pancreatic endoderm produced in vitro can generate functionally active β-cells in vivo. This was analysed by the detection for serum levels of human C-peptide at fasting, 30 minutes and 60 minutes after intraperitoneal glucose administration at the indicated post-engraftment times. One-month post implantation reduces serum C-peptide levels were detected. During the next 2 months, there was a rapid rise in human C-peptide response in most mice when measured during both FPG and stimulated plasma glucose. Three months post-engraftment, fasting levels stabilized whereas the stimulated glucose human C-peptide serum levels continued to rise (Graph 3).



Graph 3: Glucose-stimulated secretion of human C-peptide (adapted) (Kroon *et al.*, 2008).

Recently, Rezania et al. in 2014, detailed a multiple stage protocol that led to efficient embryonic stem cell differentiation into glucose-responsive insulin-producing β cells in vitro. These cells secreted insulin upon glucose-stimulation in vitro similar to that of human pancreatic β cells and reversed diabetes in STZ -induced diabetic mice within a timeframe of 3 months post-transplantation.

Together, this information provides compelling proof that hESC–derived pancreatic endoderm cells are able to differentiate into glucose-responsive, insulin-secreting β cells and aid in the reversal of diabetes in animal models. By all measures examined, these islets are physiologically very similar to pancreatic islets of humans, providing definite evidence that hES cell–derived pancreatic endoderm cells may benefit as an alternative source of islets for cell-replacement therapies in T1D patients. Further research is needed to improve the understanding of how the in vivo environment of an animal model influences cell migration and distribution, if addition of autologous vascularized cells improves engrafted PSC-derived β cells function against auto/alloimmune reaction or to determine whether or not, the non-insulin-producing cells are beneficial for hESC derived β-cell differentiation. Studies need to be conducted on the safety of the transplantation as different stages in cell progression has a significance involvement in the safety issue, since it is presumed that cells at the progenitor stage have a certain degree of residual plasticity and a greater proliferative capacity which should be significantly reduced in the differentiated mature cells, making these the safest. Furthermore, studies need to validate if an in vitro or in vivo-differentiated iPSC therapeutic approach is better due to the possibility of teratoma formation.

In the past few couple of years a lot of exertion has been directed to the encapsulation of cells in macro-devices

which are considered safe, biocompatible and permselective. Macro-encapsulation devices have been developed to provide protection to cells against immune reactions and to allow the removal of the device in case of the formation of tumours. ViaCyte, in 2014 conducted the first phase I/II human clinical trial assessing the safety, long-term effects and efficiency of a PEC-Direct product, where pancreatic progenitors derived from human ESC (named PEC-01) were encapsulated in a device and implanted in a small number of patients diagnosed with T1D. Moreover, in the year 2017, ViaCyte's PEC-Direct product has been granted approval for clinical testing by the U.S. Food and Drug Administration (FDA). Recently, ViaCyte reported good tissue coordination and increased vascularity in two patients of the ongoing clinical trial and the outcomes of the trial are expected to be revealed soon. Another device, the CPS which is a macro-device non harmful to the living tissues created to be transplanted into the subcutaneous site in T1D patients received permission for phase I/II of the clinical trials from the FDA (Cito et al., 2018). Table 5 summarizes the clinical trials which are in progress from ViaCyte. The results of the human clinical trials in progress is expected to generate valuable data and will tell us if this is the therapy for the cure of diabetes in future.

Table 5: Clinical studies of ViaCyte testing safety, tolerability and efficacy of ESC-derived PEC-01 cells encapsulated into a macro-device (Encaptra cell delivery system) (Cito *et al.*, 2018).

Identifier	Name	Cell product	Study type	Conditions	Status
Nbib2239354	A safety, tolerability and efficacy study of VC-01 combination product in subjects with type 1 diabetes mellitus	VC-01 combination product (PEC-Encap)	Phase 1/2	Type 1 diabetes mellitus, no immunosuppression	-
Nbib3162926	A safety and tolerability study of VC-02 combination product in subjects with type 1 diabetes mellitus	VC-02 combination product (PEC-Direct)	Phase 1	Type 1 diabetes mellitus, with immunosuppression	Active, not recruiting
Nbib3163511	A safety, tolerability and efficacy study of VC-02 combination product in subjects with type 1 diabetes mellitus and hypoglycemia unawareness	VC-02 combination product (PEC-Direct)	Phase 1/2	Type 1 diabetes mellitus with hypoglycemia, with immunosuppression	Active, recruiting

CONCLUSION

Diabetes mellitus is a severe health problem amongst the public, and its prevalence is increasing world-wide. The available treatments such as exogenous insulin and transplantation of purified human cadaveric islets can neither cure nor completely control the complications of this disease, which results in the loss of a great number of lives. Thus, there is an urge for the development of new treatments that provide adequate blood glucose control to minimize long-term diabetic complications. Hence, the need for alternative β cell sources of arose such as differentiation of hESCs into functionally active insulin-secreting β cells, to treat the increasing number of diabetic patients. Data from the studies used in this review supports the beneficial effect of an in vivo environment while its influence remains unclear. Moreover, effective methodologies are not yet available to protect hESC derived endoderm implants from teratoma formation or immune rejection arising from the implant. This review strengthens the fact that hESCs derived pancreatic like β islets is the most attested new source for the replacement of cells at the moment in T1D patients. In the years to come, the field of embryonic stem cell therapy will be able to transform strategies restricted to a small number of patients into a beneficial therapy readily available for a large cohort with the help of developing technology.

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