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Current progress and emerging technologies for generating extra-pancreatic functional insulin producing cells

Extra-pancreatic functional cells for diabetes management

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Abstract

Diabetes has been one of the major concerns in recent years, due to increasing rate of morbidity and mortality worldwide. The available treatment strategies for uncontrolled diabetes mellitus are pancreas or islet transplantation. However, these strategies are limited due to unavailability of quality pancreas/islet donors, life-long need of immunosuppressant and associated complications. Cell therapy has emerged as one of the promising alternative option to achieve the clinical benefits in the management of uncontrolled diabetes mellitus. Since last few years, various sources of cells have been used to convert them into insulin producing β-like cells. These extra-pancreatic sources of cells may play significant role in β-cells turnover and insulin secretion in response to environmental stimuli. Stem/progenitor cells from liver have been proposed as alternative choice which responds well to glucose stimuli under strong transcriptional control. Liver being one of the largest organ in human body and common endodermal origin of pancreatic lineages, it has been proposed one of the sources to obtain large number of insulin producing cells. The merging of nanotechnology and 3D-tissue bioengineering have opened a new direction for producing islet like cells suitable for *in vivo* transplantation in more hospitalized microenvironment. This review summarizes extra-pancreatic sources to produce insulin secreting cells with reference to emerging technologies to fulfill the future clinical need.

Key Words: Diabetes mellitus; Cell-based therapy; Insulin producing cells; Extra-pancreatic sources; Biomaterials; Tissue engineering

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Core Tip: The currently accepted available treatment strategies for uncontrolled diabetes mellitus are pancreas and islet transplantation. However, these strategies are

limited due to unavailability of quality pancreas/islet donors, life-long need of immunosuppressant and associated complications. Exogenous insulin administration is one of the clinically established options for the patients with type 1 diabetes mellitus. Liver could be an appropriate extra-pancreatic source to isolate produce number of insulin producing cells due to similar embryonic developmental origin. The furthermost need to develop more effective diabetes cell-based therapies lies in the advancements of robust sensitive micro and nanodevices for exogenous insulin-delivery.

1. INTRODUCTION

Currently diabetes mellitus (DM) and associated complications have been a major concern due to continuous increasing rate of morbidity and mortality worldwide. DM is caused by the insufficient insulin production due to autoimmune destruction of functional β-cells (insulin dependent type 1 diabetes) and insulin resistance (non-insulin dependent type 2 diabetes) and subsequent β-cell apoptosiswithin the pancreas[1]. Pancreas is an acinar gland which is a critical controller of blood glucose levels. It has both exocrine and endocrine functions. The endocrine part of the pancreas is consists of small islands of cells, called the islets of Langerhans which encompasses αcells (involved in secretion of glucagon), β-cells (involved in insulin secretion), δ-cells (involved in somatostatin secretion), epsilon cells (involved in release of ghrelin), and PP cells (releases pancreatic polypeptide). These hormones up-regulate or down-regulate depending upon the blood glucose levels and inadequate secretion of these hormones uncontrolled glycemic regulation in our circulation resulting in type 1 or type 2 DM (T2DM)[2].

The currently available treatment approaches for uncontrolled diabetes mellitus are pancreas and islet transplantation[3,4]. However, these strategies are limited due to unavailability of quality pancreas or islet donors, life-long need of immunosuppressants and associated complications. Exogenous insulin administration is one of the clinically established approach for the patients with type 1 diabetes mellitus (T1DM)[5]; however, this strategy doesn't completely control the blood glucose level, impaired insulin

release, resulting in continuous abnormal functioning of β-cells. Hence, there is an urgent need to develop clinically relevant and scalable strategy to replenish the loss of β-cell function to provide long-term recovery. Cell therapy has emerged as one of the promising alternative option to achieve required clinical benefits in both the T1DM and T2DM.

Since last decade, various sources other than pancreas derived cells have been used to generate insulin producing β-like cells[6-8]. These extra-pancreatic sources of cells may play significant role in β-cells turnover and insulin secretion in response to environmental stimuli. However, the most critical limitation with such extra-pancreatic source of cells is that they don't respond to the glucose stimuli, and don't mimic with the clinical conditions. In addition, the production of insulin producing cells (InPCs) *in vitro* as well *in vivo* requires complicated protocols and is non-glucose responsiveness. Among various extra-pancreatic sources, liver has been considered as one of the most appropriate option to isolate large number of cells having potential to dedifferentiate into InPCs due to their similar embryonic developmental origin from the endoderm. The stem/progenitor cells from liver responds well to glucose stimuli both *in vitro* and *in vivo*. Hence, liver could be one of the most suitable sources of large number of functional InPCs. This review summarizes few crucial extra-pancreatic sources which is currently proposed or in use to generate InPCs and merging of other advancements in this field which may fulfill the current clinical needs for the welfare of diabetic patients.

2. Extra-pancreatic sources of β–cells

2.1 Hepatic stem/progenitor cells

Studies have demonstrated that liver cells can be converted into InPCs under the influence of several growth factors and high glucose environment[9-11]. However, several questions remained to be addressed regarding which cell type undergo into differentiation process and the minimum cascade of genes which is essential to activate or inactivate in order to generate fully functional β–cells. An initial study by **Zalzman *et al* (2003)**[12] reported successful reversal of hyperglycemia in mice using fetal liver progenitor cells which was converted into InPCs. This study gave a boost to utilize

human fetal liver progenitors to obtain significant number of InPCs and develop relevant protocols to be applied in clinical settings. In support with the above study, **Cao *et al* (2004)**[13] also reported importance of liver cells in reversal of hyperglycemia in diabetic mice using rat liver cell line cultured in high glucose medium. However, cells failed to produce enough amount of insulin when injected *in vivo* which is comparatively very less of desired insulin content (<1%) of the native β–cells. Hence, it was assumed that primary cells are of utmost important for *in vivo* studies with further investigations.

In our preliminary study, we have demonstrated the successful production of InPCs from human fetal liver-derived EpCAM+ve stem/progenitor cells in response to glucose stimuli *in vitro*[14]. Following to this study, we also reported a cascade of transcription factors which is required to convert EpCAM+ve human fetal liver-derived stem/progenitor cells into InPCs *in vitro* under high glucose microenvironment (Figure 1). This study revealed that activation of master regulator Pdx-1 with β-cell specific transcription factor Nkx-6.1 in combination with Ngn-3, Pax-4, Pax-6, and Isl-1 is required to trans-differentiate hepatic stem/progenitor cells into InPCs under hyperglycemic challenge without need of any genetic manipulation which is crucial for its potential clinical relevance. The study also showed that amount of insulin production was much higher compared to the other developed protocols. This particular strategy has additional advantage for its clinical potential due to scalability and lack of genetic manipulation.

2.2 Embryonic stem cells (ESCs)

ESCs are derived from the inner cell mass of blastocyst. ESCs are pluripotent which has ability to produce cells present in all three germ layers including InPCs when placed in appropriate *in vitro* or *in vivo* conditions. Due to self-renewal and differentiation potential, ESCs can be used for producing large numbers of desired cell types for downstream applications. Different derivatives of ESCs have shown restoration of functional and structural benefits in injured organs/tissues[15]. Hence ESCs are considered animmense source for the generation of large number of InPCs. **D'Amour *et***

***al* (2006)**[16] demonstrated a step-wise protocol involving five stages for the conversion of ESCs into InPCs in response to a number of secretagogoues *in vitro*. Recently, **Vegas *et al* (2016)**[17] also developed multi-step protocol for converting ESCs into β-cells and achieved long-term glycemic control in immune-competent mice using encapsulation approach. However, these strategies are not clinically relevant due to non-responsive to glucose stimuli and ethical issues. Later, **Zhang *et al* (2009)**[18] developed a four-step protocol for converting ESCs into insulin/C-peptide producing cells in response to glucose stimuli. Based on the clinical applicability of this protocol, later **Hua *et al* (2014)**[19] developed a four-step protocol to convert human ESCs into pancreatic InPCs and successfully corrected the hyperglycemia in immune-deficient mice. These studies have boosted our knowledge for the experimental conversion of ESCs into functional β-cells; however, clinical applicability of these approaches are still questionable due to ethical concerns, complicated protocols and importantly these experiments were conducted in immunocompetent mice which doesn't mimic with the clinical conditions. Hence, there is still need to discover the alternative sources of human β-cells that can mimic with the clinical conditions and could be applied in clinical settings without the need of immunosuppressant. More studies are desired to obtain homogeneous population of functional human InPCs from ESCs and further to answer whether an inductive or the selective mechanism of differentiation procedure can be clinically relevant or not.

2.3 Bonemarrow-derived stem cells (BMSCs)

BMSCs are considered as one of the potential source for generating functional pancreatic β-cells[20,21]. Several studies have demonstrated experimental conversion of BMSCs into InPCs[22-25]. However, the amount of insulin/C-peptide secreted by these cells was very less compared to isolated pancreatic islets and was not enough to reverse the hyperglycemia in diabetic rats. Later several other studies were also faced similar problems with BMSCs[26,27]. Some of the results of these studies were also found non-reproducible which warrants their further investigations for clinical applicability.

2.4 Umbilical cord blood cells

Human umbilical cord blood (UCB) is known to contain stem/progenitor cell population which can be converted into different types of organ specific cells including InPCs. It is considered as one of the most appropriate source of stem/progenitor cells due to less ethical concerns and biologically waste material. Human UCB can also be readily available in sufficient amounts with low risk of graft rejection[28,29]. Few studies have successfully reported the conversion of human UCB-derived stem cells into functional InPCs by activating several crucial pancreatic transcription factors (Pdx-1, Isl-1, Pax-4 and Ngn-3) which is capable of correcting the hyperglycemia in diabetic mice[30-33]. However, for their clinical applicability more appropriate protocols need to be developed with suitable transplantation strategy to support long-term cell survival and function post-transplantation *in vivo*.

2.5 Fibroblast cells

A recent study by **Zhu *et al* (2015)**[34] demonstrated that fibroblast cells of adults or neonatals can generate precursors of endodermal lineages following the cell activation and signaling directed (CASD) trans-differentiation paradigm. They developed conditions for expansion of glucose responsive β-like trans-differentiated pancreatic endodermal cells into progenitor stage. These trans-differentiated cells have ability to control the hyperglycemia in mice and provide a new approach for the production of patient-specific InPCs for studying unresolved questions in pancreatic biology, disease modeling and drug testing strategies.

2.6 Induced pluripotent stem cells

Patient-specific cell lines can be generated using induced pluripotent stem cells (iPSCs), which can then be developed into cells of interest for disease models or cell replacement therapy. Current advances in iPSCs research have evolved several robust protocols for the production of patient specific functional β-cells. These differentiation protocols resemble with the same developmental stages ultimately targeting the final differentiated β-cells[35]. To trigger the pathways required for differentiation phases, a mixture of signaling molecules and growth factors is often utilized, with the aim of

mimicking embryonic stages of developmental. For converting somatic cells towards iPSCs, the Yamanaka factors (OCT4, KLF4, SOX2, c-MYC) are commonly employed[36]. Several studies have examined the role of iPSCs-derived β-cells in the context of T1DM[37,38]. **Maehr *et al* (2009)** for the first time demonstrated conversion of iPSCs-derived from T1DM patients into glucose-responsive functional InPCs[39]. Further, **Millman *et al* (2016)** compared differentiation potential of iPSCs-derived from diabetic and non-diabetic individuals and concluded that both the sources of iPSCs encompass almost similar expression patterns of the specific surface markers, and capacity of insulin secretion both *in vitro* and *in vivo*[40]. Despite enormous progress towards potential applicability of iPSCs-derived β-cells, there are certain technical difficulties and financial concerns related to iPSC therapy into clinical settings[41]. Although to alleviate the financial crisis, iPSCs biobanks able to match with majority of the HLA types and occasional blood types are continuously being explored. However, still the genomic instability involving chromosomal aberrations or mutations, asa well as tumor formation remain crucial obstacles towards bench to bedside clinical translation of iPSCs.

2.7 Other sources

In addition to above mentioned extra-pancreatic sources, several other source tissues have also been investigated which contain progenitor cell population that can be converted into InPCs. Among these sources, spleen[42], adipose tissues[43], blood[44], amniotic membrane and central nervous system[45] have showed β-cell specific markers during *in vitro* or *in vivo* trans-differentiation (**Table 1**). Particularly, a study by **Kodama *et al* (2003)**[42] demonstrated that injection of splenocytes can be converted into InPCs and minimizes the onset of autoimmunity. Transplantation of these cells combined with Freund's adjuvant improves diabetes in non-obese diabetic (NOD) mice. However, subsequent reports[46,47] found no such evidence which questions their clinical applicability.

3. Emerging technologies for cell transplantation in diabetes

3.1 Micro-encapsulation

Cell encapsulation technology is based on the concept of immunoisolation. Because islet cells can be effectively harvested and transplanted, encapsulation technology is quite promising for future clinical transplantation. Therefore, during the last three decades various encapsulation in different animals such as mice[48], rats[49], dogs[50], and monkeys[51]. Encapsulation technology also offers enhanced cell survival post-transplantation without the use of immunosuppressive drugs. Encapsulation technology involves an artificial compartment of semi-permeable membrane call as capsule that contains cells and allows oxygen and nutrient supply. The capsule protects cells from possible injuries due to antibodies, proteins, and the potent immune cells. Hence, capsule is also referred as an 'immunoisolation device'. Diffusion of insulin, glucose, nutrients, and oxygen across capsule has additional advantages which allows efficient glucose homeostasis. Moreover, additional intravascular devices have been designed which contains small planar or tubular diffusion chamber that is directly connected with the vascular system of host and also referred as encapsulation device[52]. This device doesn't require anastomosis after implantation, hence encompasses better clinical application compared to the intravascular device.

In a recent study of **Vegas *et al* (2016)**[17], long-term glycemic control has been achieved in immune-competent hyperglycemic mice using polymer-encapsulated human stem cell–derived β-cells. This study highlighted the decreased obscurity in successful immunoprotection against xenogenic human cell implants in diabetic mice. This report provided groundwork for future studies in autoimmune animal models using xenogenic cells transplantation with the goal of achieving long-term glycemic control and cell survival to offer insulin independence for patients with diabetes mellitus. However, this study had limitation of generating β-cells using complex long duration protocol and was conducted in immune-deficient mice. Hence, further investigations are required to prove this technology clinically acceptable using wild-type diabetic model without the need of immunosuppressant.

In similar direction, our group is also working to generate functional insulin producing β-like cells *in vitro* through trans-differentiation of human fetal liver derived

EpCAM+ve enriched progenitor cells in response to high glucose concentration (**Figure 2**). This protocol is simplified and encapsulated functional β-like cells in alginate beads (with modified protocol) have been transplanted into C57BL6 hyperglycemic wild-type mice without the need of immunosuppressant. The mice were able to restore the blood glucose level after one month post-transplantation of encapsulated cells and maintained up to 90 days (Unpublished data). In our view this is the only study, where xenogenic transplantation of human functional β-like cells has been transplanted into wild-type mice without immunosuppressant. The encouraging results of this study have showed us a path to further investigate their potential in clinical applications.

3.2 Hydrogel-based cell transplantation

Suspending viable cells in an aqueous medium of hydrogel precursors, infusing the cocktail to target areas, and stimulating cross-linking (gelation) to generate 3D gel matrices in situ are the three primary phases in cell transplantation utilizing hydrogels. Cell transplantation using hydrogel-based strategy has several crucial advantages. Hydrogels are injectable, can be used to construct cell-encapsulated gels which have a uniform distribution of cells in the transplanted space. They have water content which can be used to construct different shapes and sizes. Hydrogels can be used for cell therapy when they are constructed with limited pore size that can facilitate diffusion and metabolic wastes but prevents leakage in the cells[53]**.** Hydrogel scaffolds can be coated with encapsulated InPCs and placed at the site of transplantation[54].

Despite these advancements, using hydrogels to control the fate of transplanted therapeutic cells still poses significant obstacles including unsatisfactory cell survival rate due to their death in the new microenvironment post-transplantation. Further cells that survived in the hydrogels had uncontrolled interactions, proliferation, and differentiation. The overall therapeutic efficacy was unsatisfactory using this method. The non-autologous cells were rejected by the host immune system and the cells were strictly confined. The encapsulated cells were not able to proliferate freely, the cell viability varied for different cell types and in many cases it decreased to nearly 50%

when assessed after 10 wk. Hence, the methodologies were modified to improve the drug delivery and integration with the target tissue for successful delivery of the cells.

To improve the functioning and stability of the implanted cells dual layer hydrogels and silk hydrogels are being tested for efficient transfer of islet cells to required site. Silk hydrogels are formed using extra-cellular matrix (ECM) proteins such as collagen IV and laminin. These hydrogels were tested for cell signaling and survival by immunostaining and oscillatory theometry and found to be stable. The silk hydrogels encapsulated with islet cells in laminin had high insulin response compared with non-encapsulated cells when stimulated over 7 day period. Silk hydrogels allow co-encapsulation of other ECM proteins and cells that can ultimately used as a transplant device for treatment of T1DM[55].

3.3 Gene therapy

The generation of functioning β-cells by genetic engineering necessitates a thorough knowledge of pancreas development and a good understanding of the organ. The production of a cascade of transcriptional regulators, as well as their involvement in regulating endocrine cell fate and maturation, are important considerations for creating an artificial β-cell. Furthermore, the type of vector employed for gene delivery and the selection of suitable cell type candidates for differentiation into functional β-cells are key challenges.***3.3.1 Selecting an ideal cell type***

The ultimate aim of the gene therapy is to produce cells having ability to produce insulin and maintain blood glucose level *in vivo*. Different types of cells have been experimented since last few years' in particular pituitary cells which contain both the pro-insulin processing enzymes and the secretory granules. However, these cells were non-glucose responsive and later became glucose responsive after transfection with glucose transporter 2 and glucokinase genes. But *in vivo* production of adenocorticotropic hormone inhibits insulin function which limits its clinical efficacy[56]. Later muscle cells, liver cells, mesenchymal stem cells (MSCs) from UCB and bone marrow cells were also experimented by gene transfer technology and demonstrated the reversal of hyperglycemia in immune-deficient diabetic mice. More recently, a

combination of gene and cell therapy was opted to produce glucose responsive InPCs after retroviral transfection with Pdx-1. Transplantation of this combination reversed hyperglycemia in immune-deficient diabetic mice[57].

3.3.2 Choice of vector for gene delivery

The ideal way of gene delivery in cells rely on integrating viral vectors for sustained gene transfer into the daughter cells to provide sustained therapeutic benefits throughout the life of patient. Mainly 4 different kinds of viral vectors have been opted *in vitro* and *in vivo* models for gene delivery purposes: 1) retroviral vectors[58], 2) adenoviral vectors[59], 3) adeno-associated vectors[60] and 4) lentiviral vectors[61]. Among these, lentiviral vectors have got more popular choice for gene delivery in animal model of the diabetes. However, the clinical applicability of these strategies is limited due to viral mediated complications and resultant pancreatic trans-differentiation.

3.3.3 β-cell transcription factors

Transcription factors play significant role in determining the phenotype of β-cells. Homeobox factor Pdx-1 has been considered as the master regulator having crucial role in early development of the pancreas[62]. Hes-1 and Neurogen-3 are present in pancreatic progenitor cells and direct the respective compartmental fates through Notch signaling[63]. However, the subsequent differentiation into respective pancreatic cell lineages, a cascade of transcription factors is required to activate. Studies have reported that all endocrine cells express Neurogen-3 which activates NeuroD1 which maintains the endocrine cell differentiation program[64]. As soon as the endocrine cell differentiation program is activated, Pax-4 and Pax-6 direct the differentiation into different kinds of endocrine cells[62]. In these two transcription factors, Pax-4 is responsible for the fate of β- and γ-cells, and Pax-6 is essential for α-cell fate. More importantly, NK homeobox factors, Nkx2.2 and Nkx6.1 are responsible for driving the β-cell fate. Both these transcription factors are imperative in β-cells differentiation process. Interestingly, Nkx6.1 is also responsible for preservation of β-cell function during its interaction with Pdx-1, the master regulator and other transcription factor NeuroD1 that modulates insulin transcription. The alteration of the expression of these

transcription factors and their sub-cellular localization will alter the cellular processes related to β-cell differentiation, and cell cycle modulation and function.

3.3.4 Direct gene transfer

In relation to β-cell replacement therapy, direct gene delivery approach represents one of the potential technologies to obtain β-like cells phenotype in autologous tissues[65]. Although in past decades a lot of work were predominantly focused on direct transcription factors/genes transfer in hepatocytes, the emergence of stem cells have changed the way to look into the cells and needs further investigations due to their clinical suitability. However, before developing suitable strategy, one needs to consider the appropriate type of transcription factor for direct delivery and true conversion to functional β-cells without causing future complications.

3.4 Nanobioengineering

Nanobioengineering is an emerging field which has potential to revolutionize the diabetes treatment. Merging of nanotechnology with medical biology has given a new direction of our thoughts to create 'smart delivery' systems that can regulate blood glucose levels within the body and could produce the desired amount of insulin. Recent advancements have been made in this field and novel blood glucose measurement and insulin delivery modalities are being developed to improve the quality of life of the diabetic patients (**Figure 3**).

Using nanobioengineering approach, **earlier** developed a novel smart insulin patch can deliver glucose-responsive insulin with the help of a painless microneedle-array patch. This device is based on the glucose-responsive enzymatic mechanism which can regulate the blood glucose level in T1DM faster than the commonly used pH-sensitive formulations. In addition, it can also avoid the risk of developing hypoglycemia. Another study by **Lee *et al* (2016)**[66] showed the diversified application of grapheme-based electrochemical device to monitor the diabetes and efficient delivery of drugs transcutaneously to reduce blood glucose levels in hyperglycemic mice.

In our recent study, we have demonstrated applicability of an unique strategy that enables effective trans-differentiation of human HPCs into InPCs on 3D-nanostructured

TiO_2 substrate developed on conducting surfaces[67]. This 3D-TiO_2cellularized chip was able to reverse hyperglycemia in wild-type mice (C57BL6) when transplanted into peritoneal cavity. We also observed enhanced cell survival and insulin production, and long-term glycemic control in hyperglycemic animals where it doesn't elicit significant immunological response after ectopic transplantation of such cell-laden TiO_2 implants generated using conducting substrates. The another advantage of this approach includes sufficient amount of insulin production in very short time post-transplantation in hyperglycemic animals. Due to relatively rapid insulin production into the bloodstream, this approach is more successful in reducing the need for exogenous insulin in T1DM. Ongoing investigation has shown that by expanding the surface area of microchips, this strategy can be used to scale up the procedure for housing sufficient number of InPCs. However, more specialized 3D packaging of InPCs, as well as pancreatic exocrine and ductal cells using TiO_2 nanostructures supported by conducting substrates, would be required to construct a full pancreatic organotypic system to evolve a more effective approach of regulating hyperglycemia. Therefore, further exploration of this approach is required to achieve its real therapeutic possibility for the clinical management of hyperglycemia.

3.5 Neo-organ bioengineering

Neo-organ bioengineering is one of the most desired and promising approach which includes decellularization and repopulation of whole organ harvested from human or xenogenic sources. This approach generates a whole functional neo-organ construct for future clinical applications as a bridge therapy of organ transplantation. It offers a unique strategy of using natural organ platform to produce natural organ system using different sources of InPCs. Therefore, this particular technology can overcome the above mentioned critical concerns and has attracted a lot of attention to generate different organs including pancreas[68].

Although few recent studies have reported the use of decellularized pancreas to produce functional neo-organs for *ex vivo* insulin secretion[69-71]. However, a critical stumbling block is the lack of sufficient organ pancreata for decellularization and

repopulation. Furthermore, the mechanical integrity of the neo-pancreas formed has not been reported. The developing concept of using heterografts to create viable humanized neo-organ systems has opened up a new avenue for meeting transplant demand[72,73]. Nevertheless, due to the small number of research conducted, some important questions remained unanswered. To date, no effective decellularization and repopulation of xenogeneic spleen sources with endocrine cells has been documented. As a result, it is yet unknown if decellularized spleen can be repopulated with human InPCs and perform similarly to islets or the entire pancreas.

Our recent study has demonstrated a heterograft approach of using whole decellularized xenogenic scaffold of spleen to generate functional construct which is capable of producing desired amount of insulin in response to hyperglycemic stimulus[74,75]. In our preliminary study, we standardized a unique strategy for activating pancreatic transcription factors of EpCAM+ve enriched human hepatic progenitor cells repopulated within acellular spleen harvested from rats[74]. This findings indicates that three-dimensional, intact acellular splenic scaffolds can provide a superior microenvironment for long-term survival of cells, activation of crucial transcription factors, and trans-differentiation of hHPCs into functional InPCs. Our subsequent study demonstrated that heterograft approach developed in our previous study generates secondary neo-organoids during ectopic transplantation in diabetic rats and is capable of transporting insulin into blood stream which is essential to manage uncontrolled blood glucose levels[75]. Moreover, this study provides the first proof-of-concept for creating bio/immune compatible, humanistic insulin-producing neo-organoids, which could evolve more acceptable functional biological implantable devices for long-term diabetes management.

4. Importance of transplantation sites

The ideal choice for cell transplantation offers optimum engraftment and long-term cell function. The appropriate site for cell transplantation should pass 1) membrane drainage for the permeabilization of blood glucose and to avoid systemic hyperinsulinemia, 2) rich arterial supply, 3) minimal invasive infusion, 4) should allow

access for morphological and functional follow-up of the transplant, 5) microenvironment with maximum cell survival, and 6) immunological tolerance. Such type of transplantation sites need to be defined. Since last few years, various sites have been attempted for islet cell transplantation[76] to manage diabetes in different animal models. Among different proposed sites/route, portal vein has been the site of choice for clinical transplantation (**Table 2**). However, inflammatory reactions and low oxygen tension leads to cell loss and varied response from patient to patient. Peritoneal transplantation has advantage of immunologically privileged site, and offers enough space for housing the cells. Hence, peritoneum overcomes on the limitations of other identified transplantation sites and could be an ideal choice of ectopic transplantation. In our recent study, we have demonstrated the usefulness of peritoneal site in diabetic animal model and reported that this site helps increased proportion of animal survival and faster recovery of the normoglycemia within 30days post-transplantation without the need of immunosuppressant (Unpublished).

5. Clinical Challenges

The major challenges in diabetes cell therapy are 1) identifying clinically acceptable source of cells that can be further used to produce homogeneous population and therapeutic dose of insulin secreting cells, 2) prevention from immunological rejection post transplantation, 3) *in vivo* glucose responsiveness of transplanted cells, 4) long-term cell survival and function post-transplantation, and 5) no need of immunosuppressant. Other clinical challenges includes safety of the transplantation procedure, determining the cell delivery and engraftment efficiency using live clinical imaging system, cell delivery at the targeted site within a clinically relevant time, identification of ways to promote regeneration of resident β-cells, ease of source tissue collection and clinical grade cells isolation, and cost effectiveness of procedures. These key considerations and challenges are needed to be resolved to successfully breach the stem cell-based therapeutic possibilities for the timely management of T1DM into the clinical practice. Given to the current debate on such issues clinical applicability of stem cell-based

therapies for the treatment of diabetes mellitus is still a future goal, having great possibility.

CONCLUSION

In summary, in the next decade we expect stem cell-based therapeutic strategies in combination with nanotechnology and other potential areas of science for improved management of diabetes. Recent developments in neo-organ bioengineering and FDA-approved nanotechnology-based formulations with the success of insulin-delivery are encouraging and provide newer opportunities for diabetes treatment. In our view, the furthermost need to develop more effective microencapsulation, neo-organ bioengineering, and nanotechnology-based diabetes therapies lies in the development of robust sensitive micro and nanodevices for insulin-delivery using clinically acceptable platforms.

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SIMILARITY INDEX

PRIMARY SOURCES

1	D Gerace, R Martiniello-Wilks, B A O'Brien, A M Simpson. "The use of β-cell transcription factors in engineering artificial β cells from non-pancreatic tissue", Gene Therapy, 2014 Crossref	20 words — < 1%
2	hdl.handle.net Internet	17 words — < 1%

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