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MINI REVIEW

β cell replacement therapy for the cure of diabetes

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ABSTRACT

Islet transplantation is an important option in the treatment of type 1 diabetes. However, a donor shortage and immunosuppressant-related complications are the current major hurdles of islet transplantation. In this review, we discuss recent updates on islet transplantation to overcome these current obstacles and we share our perspectives on future β cell replacement therapy.

Type 1 diabetes is an insulinopenic disorder developed by the autoimmune destruction of β cells. Near total destruction of β cells results in hyperglycemia and subsequent diabetes-related complications (diabetic ketoacidosis, micro- and macrovascular complications). The prevalence of type 1 diabetes is increasing worldwide[1]. However, the treatment to overcome type 1 diabetes remains challenging. The mainstay of type 1 diabetes treatment has been basal/bolus insulin injection (multiple dose insulin injection, MDI) or continuous insulin infusion (insulin pump). These conventional treatments often fail to stabilize glycemic excursions affected by the lifelong variables (food, activity, stress) that patients with type 1 diabetes may encounter on a daily basis. For the past few decades, technological advancement has been made to overcome this limit. More durable or rapid-acting insulins are now available and an automated insulin infusion pump connected to a continuous glucose monitoring system has been developed. Despite these advances, recurrent hypoglycemia and glycemic variability still remain as major issues in the treatment of type 1 diabetes. The current exogenous insulin delivery still fails to replicate the physiologic function of *bona fide* β cells.

Because type 1 diabetes develops from a near total β cell depletion, β cell replenishment can be an ideal option for treating patients with diabetes. In this sense, islet transplantation remains as an ideal option for the treatment of type 1 diabetes[2]. According to the Collaborative Islet Transplant Registry (CITR) report, 1,086 patients received allogenic islet transplantation during 1999–2015[3]. After many years of experience, researchers have refined the islet transplantation protocol and have identified conditions (proper immunosuppressant use – a combination of anakinra and etanercept, IEQ ≥325,000, a recipient age ≥35, BETA-2 ≥15) for a favorable outcome[3,4]. Under the clinical islet transplantation (CIT)-07 protocol, the majority of patients with type 1 diabetes who underwent islet transplantation (87.5% in 1 year, 71% in 2 years) achieved stable glycemic control (HbA1c <7% without severe hypoglycemia)[5]. However, long-term immunosuppressant use led to a significant decline in the glomerular filtration rate and an increase in the risk of infection[5]. Another important issue is the survival of the transplanted islet graft. Importantly, 73% of recipients required an islet re-infusion[5]. In a Canadian group, the average graft survival period was 5.9 years and only 8% of patients remained insulin independent at 20 years after transplantation[4]. Similarly, C-peptide stimulation was sustained longer than 5 years after the islet transplantation in a Japanese group[6]. However, donor shortage and immunosuppressant-related complications are major hurdles for the wide use of islet transplantation. In this article, we discuss recent updates on islet transplantation to overcome these current obstacles and we share our perspectives on future β cell replacement therapy.

To overcome the donor shortage problem, stem cell-differentiated β cells and xenogeneic islets can be an alternative

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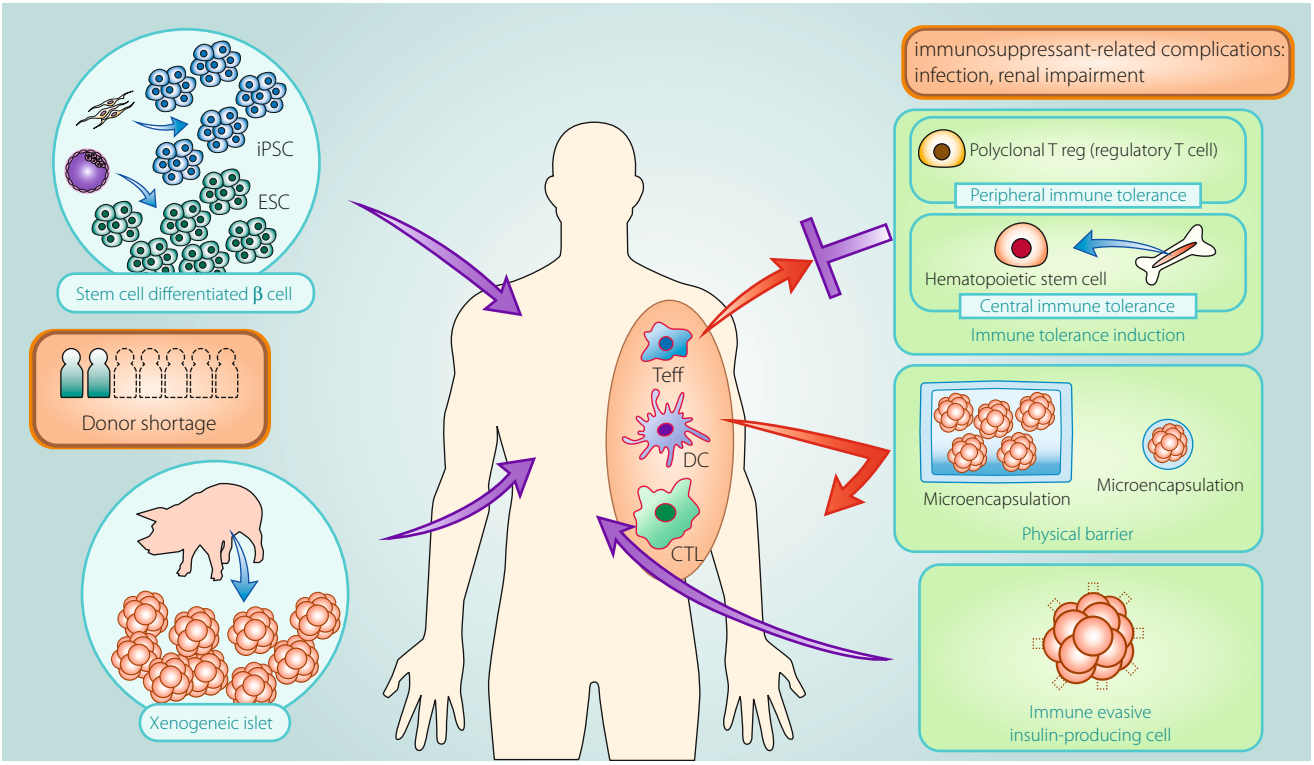

Figure 1 | Current efforts to overcome the hurdles of islet transplantation. Donor shortage and immunosuppressant-related complications are major hurdles of current islet transplantation. Stem cells (ESCs, iPSCs), xenogeneic islets are a future alternative source of islets that can overcome the donor shortage (left). Immune tolerance induction (central, peripheral), physical barriers (micro, macrocapsule), and immune-evasive insulin-producing cells are studied to evade immunosuppressant-related problems (right). CTL, cytotoxic T lymphocyte; DC, dentritic cell; ESC, embryonic stem cell; iPSC, induced pluripotent stem cell; Teff, effector T cell.

and sufficient source of islets (Figure 1). In 2014, Pagliuca *et al.*[7] demonstrated that functional insulin-producing cells can be differentiated from embryonic stem cells (ESC). After this research, more advanced differentiation methods that can differentiate ESC to β-like cells more maturely and efficiently were developed[8,9]. Based on these accomplishments, Vertex started a phase 1/2 clinical trial (VX-880, NCT04786262) in 2021. In this clinical trial, ESC differentiated β-like cells were transplanted to 17 patients with type 1 diabetes who frequently experienced severe hypoglycemia and had hypoglycemic unawareness. The β-like cells were delivered to subjects by the portal vein under immunosuppressant use and the efficacy and safety were evaluated. Recently, the early 90 day results of the VX-880 clinical trial have been announced. The patient who underwent the trial experienced an elevated fasting/postprandial C-peptide level (undetectable to 280/560 pmol/L), a decreased HbA1c (8.6–7.2%) and a reduced daily insulin dose (34–2.9 U/day)[10]. This exciting result gives hope for future islet transplantation, but long-term data with a larger number of patients are awaited. Another source of stem cells is induced pluripotent stem cells (iPSC). Like ESCs, iPSCs were shown to have the potential to differentiate to insulin-producing cells[11–13]. Patient derived iPSCs have strengths over ESCs in ethical problems and in immunosuppressant-related complications. When combined with gene-editing technologies, iPSCs-derived β-like cells can serve as a valuable resource for future precision medicine. Indeed, CRISP-Cas9 guided diabetes-related gene (SUR1, WFS1, ZnT8, INS, GLIS3) editing in iPSCs has been shown to restore impaired function in patient-derived β-like cells[14–19]. Despite these strengths of iPSCs-derived β-like cells, the low differentiation efficiency and inter-patient variability of function and yield are major hurdles. Recently, Hogrebe *et al.*[20] introduced a highly efficient iPSC differentiation protocol by targeting the cytoskeleton with latrunculin A (29.1% of NKX6.1^{+}C-peptide^{+}). These results encourage the use of iPSCs-derived β-like cells in future diabetes treatment. Further research should be followed to prove their benefits in clinical application.

Xenogeneic islets are another alternative source for islet transplantation. Pig islets are the most studied xenogeneic islet for transplantation because pigs are relatively easy to breed and raise, produce a large number of islets and their insulin is similar to that of humans. Safety is the most important issue in xenotransplantation. In pigs, porcine endogenous retroviruses (PERVs) are important pathogens as they are integrated through the pigs' whole genome. One human study reported that PERV was not detected in human type 1 diabetes

recipients even long after the pig islets were transplanted[21]. In addition, PERV, herpes virus, cytomegalovirus, and other microorganisms are potential sources of zoonosis that should be considered as well. Recently, a genetically engineered porcine heart was transplanted to a 57 year old man. The recipient survived 2 months after surgery and the medical team recently reported that the patient had been infected with porcine cytomegalovirus[22]. Thus, zoonosis is still an important issue in xenotransplantation. For this reason, the current International Xenotransplantation Association (IXA) guideline recommends the use of PERV-C negative pigs nurtured at a designated pathogen-free facility[23]. In 2016, Matsumoto *et al.*[24] demonstrated the efficacy of islet xenotransplantation in humans. In this study, microencapsulated pig islets were transplanted in eight patients with type 1 diabetes without immunosuppressant coverage. The patients who underwent transplantation experienced long-term alleviation of hyperglycemia (HbA1c <7.0% lasting over 600 days) and the restoration of impaired hypoglycemic awareness[24]. These results suggest that xenogeneic islets can be transplanted safely and effectively to humans without zoonosis. We speculate that xenogeneic islets can play a role as a bridging source of islets before stem cell-derived β-like cells can be applied clinically.

The recipient's immune response to donor islets is another important hurdle to overcome in islet transplantation. The current protocol recommends the depletion of T-cells and the use of TNF-α inhibitors during induction of the immune suppression and the use of a calcineurin inhibitor or mTOR inhibitors during the maintenance of immune suppression. This immunosuppressive regimen markedly enhanced the outcome of islet transplantation[3]. However, the long-term use of immunosuppressants impaired the renal function and increased the risks of infection in the recipients[3,5]. To overcome these immunosuppressant-related complications, the induction of immune tolerance, physical barriers, and immune-evasive insulin-producing cells have been studied (Figure 1).

Immune tolerance is a highly regulated state in which the host does not respond properly to the antigens that normally should have provoked an immune reaction[25]. Allogenic immune rejection is mediated by a CD4+, CD8+ T cell immune response. For this reason, researchers have tried to manipulate Treg cells to induce immune tolerance during the islet transplantation[26]. There is an ongoing clinical trial (NCT03444064) that infuses *in vitro* expanded Treg cells to the islet transplanted patients (peripheral immune tolerance). Successful preclinical studies were reported to induce immune tolerance by co-transplantation of bone marrow-derived hematopoietic stem cells (central immune tolerance) with allogenic islets to the recipient animals[27,28]. However, clinical data are lacking and hematopoietic stem cell transplantation-related side effects such as graft vs host disease should be considered.

A physical barrier is another option for allotransplanted islets to evade the host immune response. Micro- and macrocapsules were designed to block the entrance of host immune cells, whilst ensuring the survival of the islets. Microcapsules ideally encapsulate one islet in one capsule. Alginate is the most studied microencapsulation material. Alginate is a durable material with a low cost and high availability. Alginate was shown to be superior to chitosan, agarose, or collagen for its low immunogenicity and consistency to produce an even pore size[29]. However, several studies reported inconsistent results regarding the biocompatibility and immunogenicity of alginate capsules[30]. For this reason, constant efforts have been made by researchers to enhance the function of alginate capsules by chemically modifying their components. For example, barium-alginate cross-linked microcapsules prolonged the survival of allogenic islets up to 350 days in transplanted animals[31]. Triazole–thiomorpholine dioxide (TMTD) alginate-encapsulated human stem cell-differentiated β-like cells were able to alleviate hyperglycemia in an animal model for 174 days without fibrosis[32]. There have been several phase 1/2 clinical trials evaluating the safety and efficacy of alginate microencapsulation in humans (NCT01739829, NCT00790257, NCT01379729). Data were published on one of these clinical trials (NCT01739829) and verified the safety and efficacy of alginate microcapsules for over 600 days in eight patients with type 1 diabetes[24].

Macrocapsules encapsulate a large number of islets in chamber-like devices. Macroencapsulation has strengths over microencapsulation for they are easy to monitor and can be retrieved easily when necessary. Several devices were developed but their clinical data are unsatisfactory. The βAir artificial pancreas, developed by BetaO2 Technologies, focused highly on the oxygenation problem. The device was a disc-shaped triple-layered structure where the outside islet modules were separated from a central oxygen tank by a silicon rubber membrane. However, when it was transplanted to a human (NCT02064309), the serum C-peptide level did not change and an immunologic reaction to the device was observed[33]. PEC-Encaptra developed by Viacyte is a semi-permeable membrane structured device that allows insulins to be exported and oxygen and nutrients to be imported while isolating immune cells by the membrane pore size[29]. A phase 1 clinical trial (NCT02239354) to evaluate the safety and efficacy of PEC-Encaptra (VC01) containing ESC derived pancreatic endoderm (PEC-01) was performed. However, this study was terminated early due to insufficient function of the transplanted device[34]. The retrieved grafts were covered with fibrotic tissues suggesting that a chronic immune reaction could have hampered the graft function[34]. To overcome this limit, Viacyte developed PEC-Direct (VC02) in which the vessels directly penetrate the capsules to communicate with the contained cells. Recently, the early results of a phase 1/2 clinical trial (NCT03163511) in which subjects were transplanted with PEC-01 cells encapsulated with VC02 has been reported[35,36]. In this study, the subjects were able to produce insulin (C-peptide) in response to a meal 26 weeks after the transplantation. Moreover, insulin-positive cells with a mature phenotype (PDX1, NKX6.1, NKX2.2, IAPP, MAFA expressing) were observed, suggesting

that PEC-01 cells were able to differentiate to β-like cells in VC02 under transplantation. A minimal immune reaction to the transplanted device was observed. However, the insulin production from the grafts was not sufficient to improve glycemic indexes in the subjects, and the study was performed with the use of immunosuppressants. Therefore, clinical evidence supporting the function and efficacy of macrocapsules is still limited at present and long-term clinical data and a further improvement of the devices are required.

In addition to physically isolating islet cells from immune cells, the topological expression of immunomodulatory substances could further enhance the function and survival of transplanted islet cells. CXCL12 incorporation in alginate microcapsules prolonged the survival of islets (>300 days) with increased Tregs in the capsule[37]. Dexamethasone coating on chitosan-alginate capsule or rapamycin on polyethylene glycol-coated alginate capsule significantly decreased the pericapsular fibrosis without hampering the islet function or survival[38,39].

Genetically engineered immune-evasive insulin-producing cells can also be an attractive source of future islet transplantation. Recently, Yoshihara *et al.*[40] showed that stem cell-derived islet-like organoids overexpressing programmed death-ligand 1 (PD-L1) can ameliorate hyperglycemia in mice. In a xenogeneic source, α1,3-galactosyltransferase gene-knockout and CD46, CD59, hDAF, and CD55-expressing transgenic cross-bred pig islets were shown to be free from humoral rejection in non-human primates[41–43]. Transdifferentiated insulin-producing cells can also be an immune-evasive source of β cells. Recently, Furuyama *et al.*[44] transdifferentiated α cells into β cells by overexpressing PDX1 and MAFA. These insulin-producing cells had similar transcriptomic and proteomic features to *bona fide* β cells and were able to alleviate hyperglycemia *in vivo*. Interestingly, these transdifferentiated cells were able to secrete insulin in response to glucose stimulation and yet were hypoimmunogenic[44]. These sources are still at the research level, and further studies should be followed to validate their safety and efficacy.

Islet transplantation is a safe and efficacious treatment for patients with type 1 diabetes[2]. However, donor shortage and immunosuppressant-related problems prohibit the widespread use of islet transplantation in the clinical field. To overcome these problems, pre-clinical and clinical studies are in progress. Stem cell (ES, iPSC)-differentiated β cells, xenogeneic islets could be an on demand, unlimited source of insulin-producing cells for future islet transplantation. Immune tolerance, a physical barrier device (macro- and microcapsules), and genetic-engineered immune-evasive insulin-producing cells can be solutions for the immune suppressant-related problems. The role of β cell replacement therapy remains important and pre-clinical and clinical studies to overcome current hurdles must continue.

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DISCLOSURE

The authors declare no conflict of interest.

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