

Diabetes Mellitus-Islet Transplantation  
& Gene Therapy

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## Abstract

Type-1 Diabetes mellitus is an autoimmune disorder that comprises around 10% of the current diabetic population. The only mode of therapy for type-1 diabetes is to administer insulin. However, it is becoming very challenging to control the homeostasis between insulin and glucose levels. Islets transplantation has the potential to become a cure for diabetes. However, primary non-function of the islet graft immediately following transplantation due to apoptosis triggered by hypoxia and pro-inflammatory cytokines is one of the major obstacles for the success of islet transplantation. Gene therapy can be beneficial for improving the outcomes of islet transplantation. This review summarizes the challenges facing islet transplantation and the various viral vectors that can be used to possibly overcome those challenges.

## Introduction

Diabetes mellitus is a chronic metabolic disorder affecting millions of people worldwide. It is usually a consequence arising from environmental or genetic factors resulting in elevated blood glucose levels. It is generally categorized into two types. Type-1 or Juvenile Onset Diabetes mellitus is more common in young individuals and results due to failure of islets to secrete insulin and is mostly corrected by daily insulin injections. Insulin is an endocrine hormone released by the beta cells from Islets of Langerhans. Type-2 diabetes is more prevalent owing to lifestyle changes in developing countries, environmental factors and genetic conditions. There are several oral anti-diabetic medications available to treat type-2 diabetes. However over a period of time type-2 diabetic patients also will become dependent on insulin injections to control their blood glucose levels. It is estimated that more than 29 million people in United States are suffering from diabetes [1]. It is also further estimated that type-1 diabetes accounts for about approximately 10% of all diabetic cases worldwide [2]. Although diabetes can be managed by administering daily insulin injections, insulin does not cure the disease. The proper administration of insulin to patients can only help in decreasing the onset of developing other complications of diabetes like cardiovascular diseases, retinopathy, neuropathy, kidney problems, foot damage, Alzheimer's disease etc. Since insulin has to be administered through injections once or twice daily, patients may not be as compliant with insulin administrations as required. Also, managing the disease of diabetes requires making sure that there is a balance between the concentrations of insulin and glucose in the body. If the concentration of insulin is more (which can also happen due to improper insulin administration) it can lead to hypoglycemia that can be critical to the life of the patient if not managed properly. Similarly, if the concentration of insulin is low it leads to hyperglycemia, which is also not beneficial for the patient. Further in certain type-1 diabetic patients a condition called "Brittle Type-1 Diabetes" is developed. In this condition, patients show sudden episodes of hyperglycemia followed by severe hypoglycemia. This can further lead to convulsions, coma and death [2,3]. Also, recent projections from the Center for Disease Control (CDC) indicate that if current trends continue, there will be approximately four -fold increases in prevalence of diagnosed type II diabetes [1]. It is also estimated that almost half of adults with type-2 diabetes aren't able to control their blood sugar (glucose) numbers with their current oral diabetes medication. Hence, alternate strategies like whole pancreas transplantation, islet transplantation with novel gene therapy strategies have long been suggested as a possible alternative or cure for diabetes.

## Islet Transplantation

Human islet transplantation has the required potential to replace the islets in patient and make him/her insulin independent [4,5]. Whole pancreas transplant which has similar successes as islet transplantation, it is not recommended due to high morbidity and mortality rates except in patients who require kidney transplant [6-8]. However, a single patient generally requires islets from 3-4 cadaveric donors in order to become insulin independent or attain normal insulin-glucose homeostasis. Also, administering immunosuppressive agents, which have their own side effects, is a limitation for islet transplantation [8]. The critical challenge here is to improve the survival and functionality of islets for a longer period of time. Scientists in Canada have developed the Edmonton protocol where the transplantation of islets to a type I diabetic patients have become successful

and the patients were insulin independent one year following islet transplantation [5]. Based on the report of Collaborative Islet Transplant Registry (CITR), that patient having islet transplants between the years of 2007-2010 had an insulin independence rate of 50% three years after transplant [9,10]. It is been estimated that more than 1500 patients have been treated with islet transplantation so far and it can be considered as a viable treatment option for selective patients of diabetes [11]. However, there are few shortcomings with these therapies in that this treatment option is for selective patients who have sudden episodes of hyperglycemia, severe hypoglycemia or glycaemic lability [11]. Also, Islet transplantation is generally preferred for patients who are not responding to conventional therapies and it requires lifelong administration of immunosuppressive agents [12]. Another challenge for islet transplantation to establish as a treatment is the lack of abundant supply of pancreases from cadaveric donors.

### Gene Therapy -Viral Vectors

Gene therapy for diabetes mellitus had made tremendous progress since its inception. Some of the factors that result in primary non-function of islet grafts include immune mediated destruction of islets, apoptosis, and lack of revascularization [13]. Gene therapy strategies can be used to improve the survival and function of islets following transplantation [14,15]. Islets have a good network of blood vessels intertwined within them. This network of blood vessels is damaged during the isolation and purification of islets rendering them devoid of crucial oxygen and blood supply necessary for them to survive and function [16-18]. Also, it has been well reported that islets do not secrete insulin upon transplantation owing to their destruction by apoptosis mediated by inflammatory cytokines [19]. Therefore gene therapy approaches are focused more in the promoting revascularization and protecting the islets from apoptosis immediately after transplantation [20]. Gene therapy was initiated as a productive means to transfer of genes to human cells to treat various metabolic disorders, viral infections, autoimmune diseases etc [21]. Generally, viral vectors are more efficient in gene transfer when compared with non-viral vectors. Non-viral vectors are much safer compared to viral vectors. However, with the advent of replication deficient adenoviral vectors that can provide stable and transient expression of the genes, the safety profile of the viral vectors has been enhanced. Also, *ex-vivo* gene transfer is slowly beginning to become more significant in enhancing the outcome of islet transplantation [14,15,22-25].

Adenoviral Vectors (Adv), Adeno-Associated Viral (AAV) vectors, retro viral vectors, lentiviral vectors and Herpes Simplex Viral (HSV) vectors are the various types of viral vectors that can be employed for gene therapy [20]. Herpes Simplex Virus vectors owing to their showing only transient gene expression and their ability to elicit antiviral responses from HSV infected cells are not much used in *ex-vivo* transfer of genes to islets [20]. HSV vectors are used for gene transfer of genes such as TNF- $\alpha$  soluble receptor, interleukin-10 etc. for alleviating pain associated with diabetic neuropathy [26-29]. Retroviral vectors can provide constant expression of desired transgene as it can integrate into the host genome. However, it is not suitable for transduction of islets as retroviral vectors are not capable of transducing non-dividing cells [30]. Lentiviral vectors are modified retroviral vectors and they have the ability to transduce islet cells as they are capable of transducing both dividing and non-dividing cells

[31]. Leibowitz et al [32] in their study have shown that retroviral vectors were very ineffective in transducing islets, whereas lentiviral vectors were marginally effective. They also concluded in their study that of all the vectors they had tested adenoviral vectors showed the best transduction efficiency in islets and were very potent for transient gene expression. Jimenez-Moreno et al have recently developed a stable protocol for transducing islets with lentiviral vectors which can lead to around 80% transduction efficiency and preserving the metabolic function of the islets [33]. Lentiviral vectors have also been used to alleviate diabetes by expression of insulin in the liver of diabetic rats [34]. However, there is still a long way to go to ascertain that these vectors can be useful for islet transplantation.

AAV are relatively small with a DNA genome of 5000 bp and need a helper virus such as an adenovirus or herpes virus for transducing cells [35]. Adeno associated virus vectors capable of transducing both dividing and non-dividing cells. AAV are able to transduce the islets by binding to heparin sulfate proteoglycan receptors [36] and co-receptors  $\alpha_v\beta_5$  integrin heterodimers, fibroblast growth factor receptor type-1 and hepatocyte growth factor (c-met) [37-40]. There have been many studies using AAV vectors to express genes such as IL-2, IL-4, IL-10, TGF- $\beta_1$  [41-44] etc. and other therapeutic strategies [45-47] showed beneficial effect in the therapy of diabetes. It has been more than 50 years since the AAV vectors have been discovered and there are now close to 183 gene therapy clinical trials associated with AAV [48,49]. The major obstacles with the use of AAV are its small packaging capacity [48] which can be enlarged by using a smaller sized promoter with same efficiency [50] and newer vectors are being developed which can incorporate large transgenes [51]. The other drawback for the use of AAV vectors for gene therapy was lack of production of high viral titer which has now been solved and a number of protocols have been developed and published outlining the processes to get high yield of pure AAV vectors that are suitable for clinical trials [52]. The success of AAV vectors depend on improving the vector to avoid attacks by neutralizing antibodies, enhance its delivery and becoming less immunogenic which is also applicable for all viral vectors.

Adenoviral vectors are double stranded DNA vectors and can infect both dividing and non-dividing cells [30]. However, they do not integrate in the host genome which can be a drawback with retroviral and lentiviral vectors. They show only transient gene expression and can be considered as potent vector for *ex-vivo* gene transfer. They are capable of incorporating long genomes. Because of these properties, around 535 (21.2%) clinical trials that are currently active are based on adenoviral vectors [49]. It is interesting to note that the number of clinical trials involving adenoviral vectors has even surpassed that of clinical trials involving retroviral vectors. Adenoviruses are known to cause respiratory problems in humans. Most humans seem to have antibodies for adenoviruses which can neutralize the adenoviral vectors. This may be one of the major reasons for transient expression of these vectors. Also, to minimize immunogenicity the E1, E3 genes of the adenoviral vector genome are deleted [14,15]. By further deleting E4 gene or a part of the E4 gene of the Adv-vector most of the immune response can be minimized. We have shown that by using a bipartite adenoviral vector expressing genes Human Vascular Endothelial Growth Factor (hVEGF) / Human Hepatocyte Growth Factor (hHGF) for promoting revascularization of islets and human interleukin 1-receptor antagonist for protecting

islets from apoptosis can be beneficial in improving the outcomes of islet transplantation [14,15]. The islets transduced with adenoviral vectors Adv-hVEGF- hIL-1Ra or Adv-hHGF- hIL-1Ra when transplanted in streptozocin induced diabetic Non Obese Diabetic-Severe Combined Immunodeficient (NOD-SCID) mice, showed better control in decreasing the blood glucose levels for a longer period of time compared to islets transduced with Adv-LacZor non-transduced islets. There have been many studies focusing on using adenoviral vectors to improve the survival and function of islets following transplantation [53-58]. Human Bone Marrow Derived Mesenchymal Stem Cells (hBMSCs) have been used as gene carriers and were contrasplanted with human islets transduced with Adv-hVEGF- hIL-1Ra. This improved the glycemic control in diabetic mice [23,25]. Li R et al have used helper dependent adenoviral vectors to express neurogenin 3, betacellulin and Suppressor of Cytokine Signaling 1 (SOCS1) to promote in vivo islet neogenesis in diabetic Non-Obese Diabetic (NOD) mice. Their results showed that about 50% of the diabetic mice attained normal blood glucose levels for over 4 months [59]. The major obstacle for the adenoviral vectors to be successful is them being immunogenic. Even after deletion of E1, E3 and even E4 regions of the Adv, there is still some expression of viral genes. This ultimately leads to the Adv being active for 1-2 weeks. One way to circumvent this problem is the development of gutless Adv which can replicate only with the help of helper virus. This can enhance the expression of Adv for a long time. However, transient expression of Adv. can be beneficial for *ex-vivo* gene transfer to islets, because once the primary graft is able to survive and function in the initial few days after transplantation; it may be able to get aligned in the host.

## Conclusion

Islet transplantation is a viable alternative for the possible cure of diabetes. However, it has to overcome the challenges of use of immunosuppressive agents, non-function of islets due to immune or inflammatory cytokine mediate apoptosis. Gene therapy can be helpful in improving the outcomes of islet transplantation. It can protect the islets by promoting revascularization and protecting them from apoptosis through expression of transgenes. Scientists are even trying to increase the mass of beta cells by proliferation beta cells, convert stem cells into insulin producing cells through the processes of gene therapy [60-62]. But again the positive outcome of gene therapy of islet transplantation depends on properly controlling the delivery of the vectors and preventing host immune responses.

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