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Multidisciplinary Approaches to Islet Transplantation

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Abstract

Pancreatic islet transplantation has been a treatment option for Type 1 diabetes since 1989. The most notable refinement since then has been the implementation of the "Edmonton protocol" in the year 2000 and recently, human islet allogeneic (allo-) transplantation has attained a historical milestone in the USA with the successful completion of the National Institute of Health (NIH)-funded multicenter clinical trial. With the development of human islet isolation technology, human autologous (auto-) islet transplantation has become a clinical option for prevention of surgical diabetes following total pancreatectomy in chronic pancreatitis patients. Meanwhile, islet xeno-transplantation is developing rapidly in particular characterization of immnunosuppression regimens, development of islet encapsulation technology and availability of genetically engineered pig donors. Despite tremendous progress in areas such as pancreas procurement and preservation, islet cell isolation, culture, preservation, new immunosuppression regimens, and new biological strategies, the clinical use of islet cell based treatment, especially allo- and xeno-transplantation, is limited due to post-transplantation challenges such as the failure of primary engraftment, immune destruction of the islet graft, lifelong need of immunosuppression, and an unmet need for large numbers of islets. To address these challenges a multidisciplinary approach, encompassing bioengineering and biologic techniques, are now being applied to islet transplantation. The authors have used a bioengineering approach that includes macroencapsulation, microencapsulation, and conformal coating (islet surface modification) to protect islet cells. The biologic approach which includes anti-inflammatory /anticoagulation was successfully applied to improve early engraftment, and insulin-producing cells were generated with stem cell technology. Finally, mesenchymal stem cells were used for induction of immune tolerance and creation of a novel transplantation site. In this review we examine the application of our multidisciplinary approach and how it has contributed to the success of islet transplantation.

Introduction

A method for isolating intact islets from rodents with subsequent in vivo function after pancreatic islet transplantation in a rodent diabetes model was demonstrated by Drs. Ballinger and Lacy in 1972[1]. Nearly 2 decades later, due to advances in human islet isolation and purification, especially the development of a dedicated, "Ricordi" chamber by Dr. Camillo Ricordi in 1989[2], the first case of clinical transient insulin independence after human islet transplantation was achieved by Scharp et al. in the

year 1989[3]. Notably, a series of successful cases of allogeneic islet transplants as part of multivisceral grafts was reported by the University of Pittsburgh's team[4]. Human islet transplantation was further refined with the implementation of the "Edmonton protocol" in the year 2000[5]. Recently, human islet allo-transplantation has reached a historical milestone with the successful completion of the National Institute of Health (NIH)-funded clinical trial in the USA[6]. According to the Collective Islet Transplantation Registry (CITR) report, there have been 741 cases of islet transplant alone (ITA) and 120 cases of Islet after Kidney (IAK)/simultaneous islet kidney (SIK) transplants carried out worldwide by September 6th, 2013. In parallel with the development in human islet allo-transplantation, there have also been advances in human islet auto-transplantation (IAT). Since the first IAT was conducted by Dr. David Sutherland in 1977 at the University of Minnesota[7], there have been 562 cases of IAT reported by CITR. With the development of human islet isolation technology, human IAT has become a standardized clinical option for prevention of surgical diabetes following total pancreatectomy in chronic pancreatitis patients and pediatric hereditary pancreatitis at many centers in the USA[8, 9]. In addition to chronic pancreatitis, of islet auto-transplantation has been reported in adult and pediatric trauma cases[10] and after the resectable pancreatic[11]. Meanwhile, islet xeno-transplantation is receiving a great deal of attention worldwide due to well characterized immunosuppression regimens, development of islet encapsulation technology, and availability of genetically engineered pig donors[12-14].

Although tremendous progress has been made in islet transplantation[15], there are still obstacles to achieving successful islet transplantation in various aspects such as pancreas procurement and preservation[16,17], islet cell isolation[18,19], culture[20], immunosuppression regimens[21], and appropriate transplantation sites. Therefore, The clinical use of this treatment, especially allo- and xeno-transplantation, is limited due to post-transplantation challenges such as the failure of primary engraftment, immune destruction of the islet graft, lifelong need of immunosuppression, and limited availability of islets. To overcome these hurdles, a multidisciplinary approach to islet transplantation has been adopted that includes bioengineering and biologic techniques. In regards to the bioengineering approach the authors' work has involved the development of conformal coating (islet surface modification) of islets, microencapsulation, and macroencapsulation to protect islet cells using semipermeable biocompatible polymers. Specifically, a technique that involved layer-by-layer coating was introduced to islet conformal coating successfully making it feasible to transplant the coated islets into the recipient liver via the portal vein-as the first example. In the biologic approach, an anti-inflammatory/anticoagulation strategy using thrombomodulin (TM), and Resolvin E1, was applied to achieve improvement in early engraftment, and then insulin-producing cells were generated with stem cell technology. Finally, mesenchymal stem cells were used for promotion of islet survival[22], induction of immune tolerance[23] and creation of a novel transplantation site. In this review this multidisciplinary approach that has been applied towards the success of islet transplantation will be critically examined (Figure 1).

Bioengineering approach

Conformal coating (Islet surface modification)

The main goal of conformal coating of islets is to reduce capsule size so as to make transplantation into human subjects feasible via the portal vein. In this regard, a conformal coating method using polyethylene glycol polymer (PEG) interfacial polymerization was first reported by Hubble et al in 1997[24]. Using this method, 50-70 μm thick polymeric coatings on the surface of islets were generated. Transplantation was only possible however at subcutaneous sites rather than the liver due to the thickness. To pursue further the possibility of reducing coating thickness a layer-by-layer (LBL) polymer self-assembly was developed by several groups[25,26]. Ultimately, the author's team succeeded in developing a nanothin conformal coating, using cell surface engineering with a polyelectrolyte multilayer thin film, which was consistent with the size requirement for intraportal infusion[27-29]. In detail, the conformal coating on the surface of islets was generated by layering a biotin-labeled poly-L-Lysine (PLL)-PEG copolymer with streptavidin on the surface of islets. As shown in Figure 2, we successfully transplanted the coated islets into the recipient mouse liver via the portal vein; making this the first proof of concept for intravascular conformal coating in contrast to conventional extravascular microcapsules.

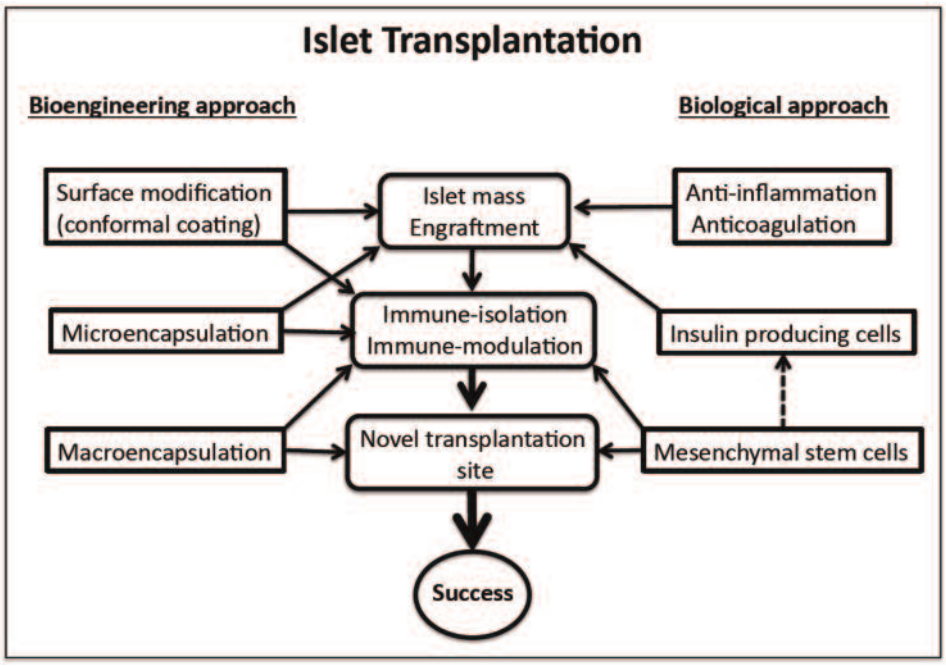

Figure 1. Overview of the multidisciplinary approach applied to islet transplantation.

Using this platform, we successfully functionalized the film with an anti-inflammatory/anticoagulation mechanism such as thrombomodulin[30,31].

Despite the significant potential of LBL strategy for islet surface modification, the main drawback of the approach is cytotoxicity of the polycationic compounds[32]. Recently Dr. Eugenia Kharlampieva (co-author) developed a non ionic LBL assembly utilizing hydrogen-bonded multilayer films based on interactions of tannic acid (TA) (a natural polyphenol) with poly(N-vinylpyrrolidone) (PVPON). Dr. Kharlampieva has successfully demonstrated this ultrathin conformal coating on the surface of mammalian islets including those derived from the rat, nonhuman primate (NHP), and human. The viability and functionality of the coated islets were maintained for at least 96 hours *in vitro*. The immunomodulatory cytoprotective properties of this coating have been demonstrated through an experiment in which this coating suppressed pro-inflammatory cytokine synthesis in stimulated bone marrow-derived macrophages and diabetogenic BDC-2.5 T cells[33]. Encouragingly, the coating material retains

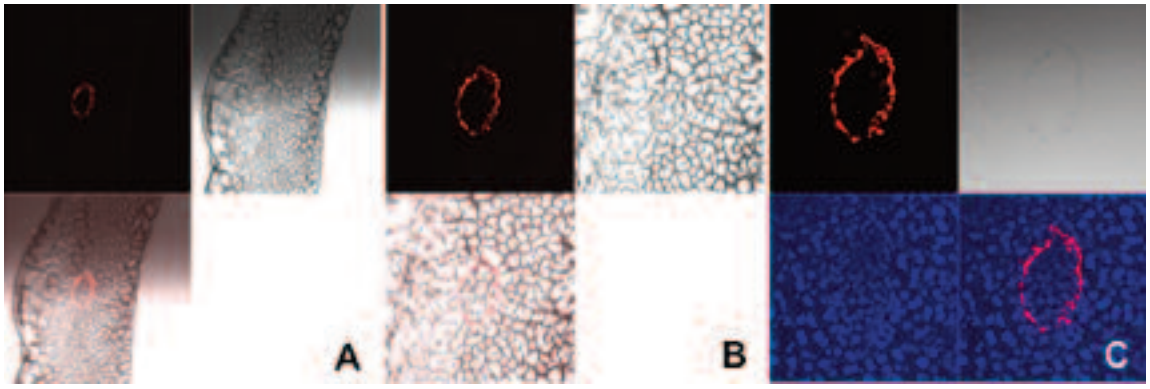

Figure 2. Confocal microscopic image of the transplanted mouse islet with Cy3 labeled conformal coating in the recipient liver. The recipient mouse liver, A: histological section from snap frozen recipient mouse (C57/BL6) liver tissue, which was harvested 15 min after transplantation of the coated B10 mouse islets into C57/BL6 STZ-induced diabetic mouse liver with a lower magnification (10x); B: the same section from A with larger magnification (20x); C: the same section from A with DAPI staining.

high chemical stability under physiologically relevant conditions and has the capability of suppressing cytokine synthesis, crucial for prolonged islet integrity, viability, and function *in vivo*.

Microencapsulation

Lim et al. first reported the microencapsulation of pancreatic islets in 1980[34]. Although various biomaterials have been used to produce microcapsules since then[35], the alginate-PLL system has been most widely investigated. The main drawback of this capsule is the poor biocompatibility due to PLL, which stimulates an inflammatory reaction on the surface of the capsules[36,37]. We introduced a membrane-mimetic thin film on the conventional alginate-PLL capsules[38]. We succeeded in coating pancreatic islets with this robust membrane-mimetic film, by in situ, visible light-mediated photopolymerization of an acrylate functionalized phospholipid assembly on an alginate-polylysine multilayer. Then we examined the performance of rat islets inside of the membrane mimetic film coated alginate-PLL capsules by transplanting the encapsulated rat islets into STZ induced diabetic NOD/Scid mice. We were able to demonstrate that the membrane mimetic film increases the biocompatibility and the bio-stability of the capsules[39]. This approach presents a promising way to increase the biocompatibility of conventional microcapsules. It also provides a convenient platform for incorporating membrane-based proteins and carbohydrates that may modulate local immunoregulatory and anti-inflammatory mechanisms, as well as other macromolecular complexes that may lead to enhanced control of interfacial transport processes.

Macroencapsulation

There are two primary types of macroencapsulation: 1) an extravascular diffusion device[40,41], and 2) an intravascular diffusion device[42]. An attractive feature of these macro-devices is that they are retrievable and/or reloadable. In the authors' early research, the focus was on the development of macroencapsulation using a Bag-type polyvinyl alcohol (PVA) device[43-45]. In order to improve the efficacy of the macroencapsulation device, we integrated fibroblast growth factor (FGF) into the device and induced angiogenesis at the subcutaneous and intramuscular sites[46-49]. We demonstrated the improvement of islet graft function in this device using in vitro and in vivo studies. In addition, we have investigated tissue engineering islets using newly fabricated chitosan sponge[50]. Dr. Howook Jun (co-author) synthesized a material called Nano-Matrix, which has potential application to macroencapsulation[51]. Dr. Jun cultured rat islets inside the Nano-Matrix and demonstrated that the Nano-Matrix enhanced the rat islet function and survival. Macroencapsulation therefore has a potential application as a convenient vehicle for islet graft at any new transplantation site.

Biomaterial related research

The authors have developed and characterized new biomaterials for application to islet transplantation published elsewhere[52-55]. For example, we biosynthesized an elastin-mimetic triblock copolymer named B9 and characterized its property with a long term in vivo study[53,55]. This triblock copolymer B9 contains identical hydrophobic endblocks with $(IPAVG)_4(VPAVG)]$ repeat sequences, separated by a central hydrophilic block with repeating units of $(VPGVG)_2(VPGEG)(VPGVG)_2]$. This structure of B9 designed by our group has a unique nature, inverse transition temperature, and self-assembly through hydrophobic interactions to form thin films, fiber networks, or vesicles. In our long term in vivo study, we monitored the dimension change of the cylinder-shape material using MRI imaging analysis, which was implanted at the mouse subcutaneous site. We found that the self-assembling, recombinant elastin-mimetic triblock copolymer elicited a minimal inflammatory response and displayed robust *in vivo* stability on mice for periods exceeding 1 year[53]. We examined the stability and biocompatibility of this material without islets in this study.

Biolological approach

Anti-inflammatory and anticoagulation

Although anti-inflammatory and anticoagulation approaches have been applied for improving islet engraftment, especially in *in vivo* studies on islet xenograft, since the late 1990s[56], these approaches have not received the clinicians' attention until recently. The application of the "Edmonton protocol" [5] comprising a glucocorticoid-free immunosuppressant regimen and a large dose of islets did not result in good long-term clinical outcome. The protocol was developed to avoid the diabetogenic side effects of glucocorticoid included in conventional immunosuppressant regimens[57]. Conversely, Dr. Bernard Hering demonstrated excellent results from

a single-donor islet transplant with Etanercept in the 2005[58] implying that the anti-inflammatory approach plays a critical role in human islet transplantation. The theoretical background to this approach is related to the instant blood mediated inflammatory reaction (IBMIR). This reaction was first well illustrated by Bennet et al in 2000[59] and discussed by Citro et al in a recent review summarizing anti-inflammatory strategies used to enhance islet engraftment and survival[60]. Our own focus has been on TM and our colleague Dr. Xue-long Sun (co-author) has complied all the different domains on recombinant TM for pharmaceutical, biomedical, and cell transplantation[61]. After confirming the anticoagulation and anti-inflammatory effect of TM in a *in vitro* setting by culturing human islets with human blood from an allogeneic donor using a heparinized loop model, we achieved excellent results with intra-liver islet transplantation in mouse syngeneic and allogeneic models (Figure 3)[62, 63]. We demonstrated that TM treatment significantly improved early islet engraftment with down regulation of both coagulation (i.e., marked reduction in intraportal fibrin formation) and inflammation (i.e., marked reduction in neutrophils infiltration and IL-1β, and TNF-α) pathways. We showed that this innovative strategy creates a local anti-inflammatory microenvironment, which limits the graft-triggered coagulation cascade, inhibition of thrombin formation and reduction in platelet activation, and leukocytes recruitment. In addition, we examined several potential biological reagents in this category. For example, we synthesized dendrimer-like poly (ethylene oxide) (PEO) glycopolymers and demonstrated their anti-inflammatory property in a mouse peritonitis model[64]. Furthermore, we identified that reconstitution of CD39 in liposomes restores thromboregulatory function by amplifying nucleoside triphosphate diphosphohydrolase activity[65]. At the same time, we demonstrated that Resolvin E1, which mediates an active process by specific signals in the resolution of acute inflammation[66], improves early and prolonged function of allograft islets (unpublished). Additionally, we were able to functionalize the conformal coating with these reagents[31].

Induction of insulin producing cells from somatic cells

Sources for generation of insulin producing cells includes differentiation from embryonic stem cells, proliferation of existing adult beta cells, derivation from putative adult progenitors/stem cells, and reprogramming of non-beta cells to beta cells[67]. With the recent advances in the field of cellular reprogramming, an approach based on direct reprogramming to replace or supplement islet transplantation is foreseeable. In 2008, Dr. Douglas Melton's group demonstrated that mouse pancreatic exocrine cells can be reprogrammed to insulin-producing cells *in vivo* via viral transduction of several genes encoding pancreatic and beta cell transcription factors, namely, Ngn3, Pdx1, and MafA[68]. The advantage of this strategy is two-fold. First, it circumvents the issue of donor tissue scarcity. Second, because the reprogrammed cells

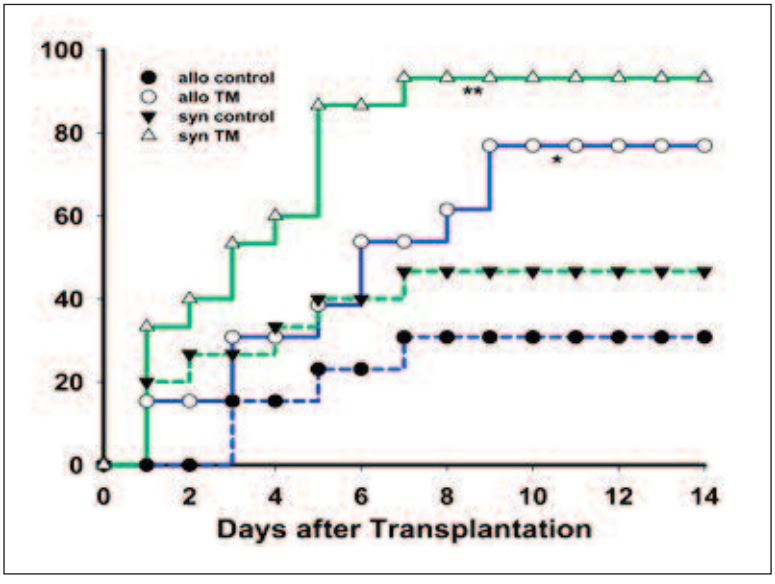

Figure 3. Thrombomodulin improved the engraftment of both syngeneic and allogeneic islet grafts after intraportal transplantation. Pancreatic islets from B10 and B6 mice were transplanted into male B6 mice that were rendered diabetic by streptozotocin (200 mg/kg IP). Diabetic animals received either 250 islets alone (n = 14-15); or 250 islets along with TM/lipid vesicles (n = 12-15) by intraportal injection. In the group receiving TM/lipid vesicles, TM at a dose of 1mg/kg was administered IV via the jugular vein 2 minutes prior to islet infusion. Serial blood glucose levels were measured and conversion to euglycemia was defined as glucose levels < 200 mg/dL for > 2 consecutive days (*; **: $p<0.05$ by Chi Square analysis).

are derived from the patients' own cells, they will not generate an immune response. Currently, Dr. Yong Zhu's group (co-author) is studying a combination of transcription factors to reprogram hepatocytes to beta cells based on the principles of gene regulatory networks. Also for practical considerations, delivering the reprogramming factors in the form of transducible proteins instead of virus-based gene vectors is under study. Cellular reprogramming-based approaches may also be used to supplement islet transplantation. Theoretically an extra step can be added to the standard total pancreatectomy with islet autologous transplantation (TP-IAT) procedure to increase beta cell mass for instance by transforming pancreatic exocrine cells, which are destroyed and discarded in current, practice to beta cells. In the clinical setting after the islets are separated from pancreatic exocrine cells in the processing laboratory and returned to the operating table for IAT, the remaining mass of exocrine cells could be cultured and treated with the cell-reprogramming protein combination. The newly created beta cells can then be purified and injected back to the patient through the portal vein one week after the initial TP-IAT. This study is at the proof of concept stage.

Creation of a new transplant site with mesenchymal stem cells (MSC)

The liver is currently the site of choice for clinical islet transplantation despite being far from ideal. Significant early loss of the islet graft results from low oxygen tension and IBMIR at the site and procedure related complications such as hemorrhage and thrombosis are major drawbacks of this site. Many investigators have been involved in searching for an optimal site for islet transplantation; up to now, islets have been transplanted experimentally into the portal vein, kidney capsule, spleen, pancreas, peritoneum, omentum, gastrointestinal wall, testis, thymus, bone marrow, vein sac, anterior chamber of the eye, cerebral ventricles, subcutaneous space, intramuscular space, and fat pad[69]. In the clinical setting, Maffi et al recently demonstrated the first unequivocal example of successful engraftment of autologous islet graft in bone marrow in humans[70]. Closely allied to this other investigators and clinicians are exploring the potential of mesenchymal stem cells (MSC) in the islet transplantation field. It has been shown in animal models that MSC have immunomodulatory and angiogenic effects as summarized by Sakata et al in 2011[71]. Motivated by these findings, we have studied the creation of a novel immunoprivileged transplantation site using both adipocyte and bone marrow derived MSCs. Theoretically, there are two major avenues of delivery of islets on a physiological basis, the intra-portal vein system and extra-portal vein system. In the authors' opinion, the omentum pouch and gastrointestinal wall are representatives of the intra-portal vein site and bone marrow and subcutaneous space are representatives of the extra-portal vein site. While so many alternative sites are under experimental investigation, Dr. Hongjun Wang (co-author) proposed a practical clinically applicable solution to improve early engraftment of the autologous islet graft in the liver by co-transplantation of bone marrow derived MSCs. Dr. Wang's proposal has been funded by the NIH (1R21DK099696-01) and is in progress. We strongly believe that this approach is a short cut to improve the clinical outcome of human islet autotransplantation at present and potentially human islet allo-transplantation in the near future.

Gene therapy

Concomitant with the current boom in stem cell research, gene therapy has regained attention from researchers and clinicians and particularly the potential for producing robust islets by delivering anti-apoptosis genes such as Akt1 into beta cells. Akt1/Protein Kinase B is the direct downstream target of PI3 Kinase activation, and has shown potent anti-apoptotic and proliferation-inducing activities. In this regard, Dr. Hongju Wu (co-author) created a fiber-modified infectivity enhanced adenoviral vector, Ad5RGDk7, and delivered it into a rat insulin promoter (RIP)-driven CA-Akt1 and hence in to the beta cells in the human and rodent islets[72]. Dr. Wu demonstrated that CA-Akt1 is effective in promoting beta cell survival and proliferation *in vitro* and it offers a beneficial bystander effect against streptozotocin-induced diabetes. This approach opens a door to a new field for diabetes prevention and treatment. In addition, Dr. Wu reported the regeneration of pancreatic non-beta endocrine cells in adult mice following a single diabetes-inducing dose of streptozotocin[73]. The study demonstrated that adult alpha and delta cells could be regenerated by both self-duplication and regeneration from endocrine precursor cells. Interestingly, Dr. Weir's group reported exocrine regeneration but no evidence of beta-cell regeneration in a pancreatic duct ligation rat model after almost complete beta-cell loss[74]. Coincidently,

both groups noted that there was no evidence of beta-cell regeneration in their respective beta-cell depletion animal models despite the positive findings in cell regeneration.

Conclusions

Islet transplantation is the perfect platform for the application of a multidisciplinary approach. As we have illustrated, bioengineering and biologic approaches (Figure 1) are essential for success of islet transplantation. The authors' experience covers the whole gamut from bench to bedside. In the bioengineering approach, we covered islet surface modification utilizing advanced techniques and novel knowledge. This approach is in the proof of concept stage now. Our research on microencapsulation, is commonly applied to islet xeno-transplantation in clinical trials and macroencapsulation is an attractive alternative for the creation of optimal transplantation site. In the biologic approach, we did not address any immunosuppressant regimen in this review since this is under intensive study by investigators in the immune tolerance network in the United States (www.immunetolerance.org) and new immunosuppressant regimens are under investigation in the NIH funded clinical islet transplantation (CIT) programs (http://www2.niddk.nih.gov/Research/ ScientificAreas/Immunology/Transplantation/CITT.htm). We would however like to emphasize the importance of anti-inflammatory and anticoagulation strategies by highlighting our contribution in this regard. In the generation of insulin producing cells, there are several approaches based on the source of the original cell; we are mostly interested in the bone marrow derived MSCs due to accessibility in human subjects and perhaps having the best potential as noted in the literature[75]. As for the application of MSCs, we are focused on three main properties; immunomodulation and angiogenesis besides insulin producing cell generation. Ultimately, we believe that utilizing this multidisciplinary approach gives us the best possibility of success in islet transplantation.

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Conflict of Interests

The authors have no financial of interest.

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