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Engineering a Local Microenvironment for Pancreatic Islet Replacement

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Abstract

Intraportal islet transplantation has emerged as a promising treatment for type 1 diabetes mellitus (T1DM). Nevertheless, long-term efficacy has been limited to a marginal number of patients. Outcomes have been restricted, in part, by challenges associated with the transplant site, poor vascularization, and disruption of the native islet architecture during the isolation process. Engineering a biomaterial platform that recapitulates critical components of the pancreatic environment can serve to address these hurdles. This review highlights the challenges and opportunities in engineering 3-D niches for islets, specifically: the importance of site selection; the application of scaffold functionalization to present bioactive motifs; and the development of technologies for enhancing implant nutritional profiles. The potential of these novel approaches to improve islet engraftment and duration of function is discussed.

Introduction

Type 1 Diabetes Mellitus (T1DM) results from the autoimmune destruction of β -cells within pancreatic islets of Langerhans. While exogenous insulin, supplemental agents, and dietary regulation can provide reasonable management of blood glucose and mitigate secondary complications, only the replacement or regeneration of β -cells can offer physiological glycemic control. Clinical Islet Transplantation (CIT) represents a viable treatment option for T1DM. The current procedure involves the infusion of allogeneic cadaveric islets into the hepatic portal vein of the recipient. While most CIT recipients exhibit stable glycemia, decreased hemoglobin A1c, and elimination of hypoglycemia for multiple years, long-term (> 5 years) insulin independence has been achieved in only a subset of patients [1].

Failure of the graft in the long-term can be explained, in part, by the chosen transplant site. Infusion of the islets into the hepatic vasculature leads to an instant blood mediated inflammatory reaction (IBMIR), resulting in islet necrosis. Delay in graft revascularization and the ensuing hypoxia further exacerbate islet loss. Moreover, the higher concentrations of

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drugs and toxic byproducts in the hepatic microenvironment can dampen the function and engraftment of the transplanted islets [2].

While the initial success of islet transplantation highlights the potential of these cells to treat T1DM, the substantial loss of islets post-transplantation directs current research towards engineering superior transplant microenvironments. Ideally the appropriate 3-D niche should be tailored to the specific requirements of islets, customized to the natural, unique extracellular matrix architecture of the native pancreas. To meet the high oxygen demand of the islets, it can be supplemented with oxygen prior to competent vascularization, while simultaneously directing and accelerating the revascularization process. A defined engineered microenvironment can also provide a platform for local modulation of the inflammatory and immunomodulatory process, thereby reducing the systemic burden. Finally, a defined transplant site permits noninvasive assessment and the safe retrieval/replacement of the graft, if necessary. This review provides a description of the current research in engineering an optimal transplant site (Figure 1) and how a combinatorial approach can lead to dramatic improvements in the long-term success of islet transplantation.

Selecting the Optimal Engraftment Site

Engineering the optimal transplant microenvironment should begin with selecting the appropriate implant location. As evident from the native pancreas, vascular density and portal drainage of the site should be important factors. The sensitivity of islets to nonspecific inflammation during the peri-transplant period, with inflammatory reactions and oxidative stress promoting β -cell apoptosis, calls for the selection of a site with minimal inflammatory reactivity [3,4]. Logistical factors, such as ease of implantation and adequate space of housing device, should also be taken into consideration.

Numerous locations have been explored as alternate islet implantation sites, with variations in efficacy and reproducibility (for a more comprehensive review see [5]). Clinically relevant sites include: intraperitoneal; intramuscular; subcutaneous; omentum; and venous sac. The intraperitoneal cavity is amenable to greater transplant volumes, making it a popular site for encapsulated islets; however, it is challenged by lower oxygenation, limited capacity for revascularization, and delays in glucose responsiveness. Transplantation within striated muscles has shown promise as an alternate transplantation site, with recent clinical efficacy using autologous islets [6]. Its high oxygen tension, as well as rapid revascularization, makes the intramuscular location a favorable environment for islets; however, care must be taken in the implantation procedure to minimize clumping and mechanical stress. The subcutaneous space is a highly desirable location due to ease in accessibility and transplantation. Its potential has been limited by mechanical stress and inflammation, with suboptimal vascularization and systemic circulation drainage [7], although recent approaches using devices and/or biomaterials to develop it as a more favorable site are promising [8,9]. The omentum shows potential as a transplant site, whereby the tissue can be folded or wrapped to create a transplant pocket, permitting flexibility in transplant volumes [10,11]. It is well vascularized with portal drainage, and has demonstrated properties for enhancing the graft microenvironment, such as controlling the spread of inflammation, and promoting reconstruction and tissue regeneration [12•]. A recent report by Kakabadze *et al.* explored the potential of an isolated venous sac, nourished by its vasa vasorum, for transplanted islets [13•]. Results in rat isografts were highly promising, with improved efficacy compared to the intraportal site. Alternatively, several locations have been investigated for their immuno-privileged properties, with recent works of interest in the lymph node [14•] and the anterior chamber of the eye [15]. While likely not immune-privileged per se, these sites may provide a less inflammatory microenvironment.

Overall, careful consideration of the properties of the graft site is a critical component for optimal islet engraftment and function.

3-D Scaffolding

In their native environment, islets are embedded within extracellular matrix (ECM) proteins, typically composed of interstitial matrix and basement membrane (BM) proteins, predominated by collagen type IV, laminin, and fibronectin (for extensive review see [16•]). These dynamic 3-D structures play an instructive role in islet survival, function, and proliferation. α -cell-ECM interactions also play a critical role in the activation of NF- κ B signaling, a critical pro-inflammatory regulator [17]. Moreover, BM proteins influence endothelial cell attachment and migration [18], thus facilitating vascular network growth. Isolation procedures, however, disrupt this microenvironment, resulting in islet apoptosis via an anoikis-like pathway [19].

The introduction of a 3-D platform can provide a spatial surrogate to the isolated islets, with the benefits of islet distribution and mechanical protection. Architectural properties, such as porosity and pore size, play an important role in the design of these platforms. While microporous designs, which prevent cellular migration into or out of the implant, have the desirable property of dampening immune attack, the engineering of immunoisolating, micro-scale devices is greatly challenged by diffusion length scales. Macroporous scaffolds, characterized by pores larger than 50 μ m, can provide an open environment for housing islets. This open framework supports efficient nutrient delivery and waste removal, while permitting infiltration of host cells within the implant. This accessibility for cellular infiltration facilitates the deposition of host ECM and the formation of an intra-graft/intra-islet capillary network. Various biomaterials have been proposed for creating these scaffolds (for a comprehensive review see [20]). Synthetic polymers are commonly employed, due to their stability, reproducibility, and ease of functionalization. A popular choice for biodegradable macroporous scaffolds is poly(lactic-co-glycolic acid) (PLGA). The biodegradability of the PLGA can promote host integration and ECM formation. Gibly *et al.* demonstrated the ability of a macroporous PLGA scaffold to improve islet survival and engraftment in both rodent and porcine models, while allowing for cellular infiltration and revascularization [21]. Additionally, a macroporous scaffold made from Ethisorb, a composite degradable polymer made from a blend of polyglycolic acid (e.g. VICRYL) and poly-p-dioxanone (PDS), was shown to be effective in both nonhuman primate and dog allografts [22•,23]. Alternatively, biostable scaffolds can illustrate similar outcomes, such as the one recently reported by our group using polydimethyl-siloxane (PDMS) [24]. Islet viability within these PDMS macroporous scaffolds was enhanced under low oxygen culture conditions, when compared to microporous scaffolds of comparable dimensions. Furthermore, stabilization of glycemia and independence from exogenous insulin was observed in both rodent and nonhuman primate models [24,25].

The introduction of proteins or ligands responsible for cell-ECM or cell-cell interactions onto the base 3-D material scaffold can further minimize islet anoikis by recapitulating their native microenvironment. The significance of α -cell-ECM interactions has been highlighted heavily in the field via culture of islets atop surfaces coated with selected BM proteins [26,27]. When incorporated within 3-D matrices, such as the poly(ethylene glycol) (PEG)-based hydrogels utilized by Weber *et al.*, reduction of apoptotic pathways and improved glucose-stimulated insulin-secretion, for both a β -cell line and murine islets, was observed [28,29]. In translating this *in vivo*, Salvay *et al.* showed a decreased mean time to euglycemia and improved engraftment, when embedding islets in a PLGA macroporous scaffold functionalized with BM proteins, specifically collagen IV [30]. Alternatively, the selected presentation of specific cell adhesion motifs of importance to β -cells may provide a

more elegant means to functionalize the scaffold framework, avoiding immunogenicity and sourcing issues associated with incorporation of full matrix proteins. For example, Lin *et al.* evaluated the use of a PEG-based hydrogel functionalized with EphA–ephrinA fusion proteins to mimic native cell-cell binding [31••]. Encapsulation of β -cells, either derived from a cell line or dissociated from murine islets, within these biomimetic hydrogels resulted in enhanced survival and function, Figure 2. Another approach explored peptide amphiphiles containing arginine-glycine-aspartic acid (RGD), in addition to an matrix metalloproteinase-2 (MMP-2) sensitive sequence, for β -cell encapsulation [32]. Peptide hydrogels resulted in improved retention of glucose responsiveness and islet morphology in culture. While efficacy *in vivo* has yet to be evaluated, 3-D matrices functionalized with bioactive motifs designed to facilitate ECM-cell or cell-cell interactions can provide a powerful platform to enhance transplant outcomes by preserving differentiated function and minimizing apoptosis.

Of note, several innovative approaches have sought to utilize these 3-D structures as bioactive platforms to direct host responses, such as inflammation, either through incorporation of supplemental cells or through the tethering of bioactive agents. For example, ethylene carbodiimide (ECDI)-fixed splenocytes were co-localized with rodent islets within macroporous PLGA scaffolds prior to transplantation in an allogeneic rodent model, whereby reversal of diabetes without the need of immunosuppression was observed [33••]. Protection was evidenced by an increased accumulation of Treg cells and a reduction of interferon- γ (INF- γ), a pro-inflammatory cytokine. Elsewhere, researchers functionalized hydrogels with interleukin-1 (IL-1), an anti-inflammatory peptide, for the local protection of encapsulated islets from immunoreactive cells [34]. Results showed improved protection of encapsulated islets embedded within these bioactive surfaces when exposed to pro-inflammatory cytokines. Thus, localized 3-D microenvironments provide a potential for biomimicry that can recreate the spatial architecture and environmental signaling present in the islet's native microenvironment.

Oxygenation and Vascularization

Insufficient oxygen tension is one of the major obstacles for the success of cellular based constructs, particularly when composed of highly metabolic cells, such as pancreatic islets. While islets comprise about 1% of the pancreas, they consume approximately 15% of the blood flow [35]. Not only is the oxygen consumption rate for pancreatic islets elevated compared to many other cell types, they are also susceptible to functional impairment at moderate oxygen tensions [36]. Proper oxygenation during the initial post-transplantation period plays a critical role in islet engraftment and viability, as islets, stripped from their natural microvasculature during isolation, rely solely on diffusion to obtain adequate nutrients and oxygen. Hypoxia negatively impacts islet survival, mainly through the stabilization of hypoxia inducible factor (HIF)-1 and the subsequent activation of its target genes, which lead to a cascade of events that terminate in islet apoptosis [37]. In addition, HIF-1 has been shown to result in impairment of glucose responsiveness [38,39], which may further delay islet revascularization [40•]. While prompt re-vascularization of islets grafts is critical for transplant success, complete prevention of hypoxia exposure is more desirable. This is a particular challenge for extrahepatic sites, where these initially avascular implants are plagued with substantial nutrient delivery problems, leading to central necrosis and sub-optimal performance. Two promising approaches seeking to address this challenge are: *in situ* oxygen generation and revascularization guidance.

In situ oxygen generation is an attractive approach to improve oxygen tensions in the transition period from implantation to the formation of a functional vascular bed. The ideal oxygen generator must ensure adequate and stable oxygenation for the duration of the

revascularization process. It should not drastically affect transplant size, the transplant procedure, or require multiple surgeries. While various prototypes have been developed, more recent publications have employed oxygen chambers or hydrolytically reactive solid peroxides, Figure 3. Ludwig *et al.* developed an immunoprotective, macrochamber device containing an oxygen tank, which is refreshed daily via an external port [41]. Islet-loaded macrochambers, retrieved 13 days post-transplant from an allogeneic porcine model, demonstrated a 50% increase in islet oxygen consumption rate (OCR) over controls. Moreover, in a rat model, this device was found to improve both islet OCR and insulin secretion levels, when islets were pretreated with growth hormone releasing hormone [42•]. Recently, our group reported on the development of a hydrolytically reactive, oxygen generating biomaterial, based on the encapsulation of solid calcium peroxide [43]. The material was capable of generating oxygen over 6 weeks in culture, while co-culture with cells or rat pancreatic islets mitigated hypoxic-induced cell death when cells were cultured under hypoxic conditions.

An alternative approach to alleviate hypoxia during islet engraftment is improving the efficiency and competency of implant vascularization. Given that the formation of a functional vascular network is an intricate process, engineering a microenvironment that efficiently coordinates and directs these pro-angiogenic cells and factors is a complex task. Interventions that focus on the delivery of pro-angiogenic cues must address issues associated with pharmacokinetics, while approaches that target pro-angiogenic cells must consider cellular interactions, delivery, and nutritional burden. One recent publication explored the use of ultrasound-targeted microbubble destruction to deliver the gene for human vascular endothelial growth factor (VEGF) to the host liver prior to intrahepatic infusion of human islets in a diabetic nude mouse. Enhanced VEGF secretion in the liver was detected, resulting in significant improvement in islet engraftment and efficacy [44]. Additional strategies include the use of pro-angiogenic cells, such as mesenchymal stem cells (MSC), as modulators of the revascularization process, which has proven to increase vascular sprout formation, intra-islet vascularization, and graft efficacy [45,46]. Moreover, the transplantation of MSCs with islets may have the additional benefit of immunomodulation, as recently observed in rodent and nonhuman primate models [47–49].

The delivery of pro-angiogenic factors to the local site is a common means used to initiate and hasten the revascularization process. The use of pro-angiogenic polymers, i.e. the combination of angiogenic growth factors (GFs) and biomaterials, is an attractive approach that has shown enhanced therapeutic effect over delivery of GF alone (for review [50]). Of recent interest are hydrogels incorporated with both cell matrix and growth factor binding sites. Martino *et al.* has published on the use of a system comprised of: fibrin network; angiogenic growth factors (PDGF-BB and VEGF-A); and a recombinant fragment of fibronectin (FN) containing binding sites for fibrin, integrins, and GF [51••]. This system has been shown to provide potent synergistic signaling and morphogenesis between 5 1 integrin and GF receptors, when their binding sites are proximally presented in the same polypeptide chain. This synergy translated to enhanced regenerative effects in the treatment of chronic wounds in a diabetic mouse model. A similar presentation was engineered using synthetic polymers, such as the PEG-based gels developed by Phelps *et al.*, where resulting bioactive hydrogels demonstrated enhanced vascularization and functional outcomes in a hind-limb ischemia model [52]. This approach has recently shown enhanced benefit for islet transplants in a syngeneic murine model [53]. For islet applications, the group led by Samuel Stupp has explored the use of heparin-binding peptide amphiphiles, doped with VEGF and FGF, to house islets. Incorporation of these GFs resulted in an increase in islet endothelial cell sprouting, improved viability and glucose stimulation indices, and enhanced efficacy in islet transplants within a syngeneic mouse model [54•,55].

Future Directions

Even with the development of an ideal islet transplant site, whereby islets optimally engraft and exhibit superior long-term function, a significant obstacle in clinical translation is the requirement for systemic immunosuppressant or anti-rejection drugs. With the goal of dampening overall immune activation, systemic immunosuppression has a number of crucial disadvantages, such as increased susceptibility to disease, infection, and cancer. Given that current treatment options exist for T1DM, this approach would be restricted to only severely diabetic patients, where the benefits of optimal glycemic control outweighs the risks of systemic suppression of the immune system. Further disadvantages of anti-rejection therapy include their tendency to impair islet revascularization and function, as well as the fact that current immunotherapy options are unable to completely abrogate autoimmune attack [56]. Thus, if innovative immunoprotective or immunomodulatory strategies are developed that can selectively decrease immune response to islet allografts, without imposing significant side effects to the patient, the clinical translation of islet transplantation would be significantly accelerated.

With this grand challenge, it is critical that immunomodulatory strategies are converged with optimal implantation microenvironments to ensure success. While multiple researchers are exploring the potential of immunoprotective biomaterials or devices for dampening immune attack, all too often these innovative immunomodulatory approaches are employed using devices or implantation sites that limit islet viability and function. For example, the implantation of immunoisolating devices within the subcutaneous site, which has unfavorable inflammatory responses and nutrient profiles, can impair appropriate assessment of the device's potential. Further, the confinement of highly metabolically active islets within devices at loading densities substantially higher than native tissues, likely results in undesirable nutrient profiles. As such, testing of immunomodulatory devices or polymers should be employed within transplantation sites deemed favorable to generalized islet transplantation. Approaches should minimize metabolic loading, as well as decrease void space imposed by the material, to ensure clinical applicability and optimize nutrient profiles within the device. Convergence of immunoisolating strategies with bioactive polymers capable of mitigating islet anoikis might also prove highly beneficial to enhancing islet survival, as well as the use of pro-angiogenic agents.

Poor clinical outcomes for immunoisolating prototypes to date may also be due to the “all or nothing” dogma regarding the use of anti-inflammatory or immunomodulatory therapy. While this approach may be the most desirable, the testing of immunoprotective barriers in the complete absence of immunotherapy, particularly for xenografts, sets a high bar for efficacy. Alternatively, the complementary effects of low dose and/or short-course immunotherapy and immunoprotective materials/devices, within optimal transplant sites, may be the most efficient means to provide an alternative approach to the current clinical paradigm. The use of immunosuppressive agents during the initial engraftment period, or long-term at low doses, may prove to be highly effective at decreasing the immune burden, without imposing significant or long-term risks, which would result in a treatment alternative that may be highly desirable to many T1DM.

As a final note, the unique challenge of autoimmune responses to the allogeneic beta cells is a significant obstacle in ensuring long-term survival of these cells. Recent publications on the use of engineering nanoparticles or peptides to decrease autoreactive clones could be highly complementary to dampening potent autoimmune attack during islet transplantation [57,58]. While completely abolishment of autoreactive clones may be a weighty obstacle, there is potential that these methods may serve as a potent combinatory strategy, when

combined with the approaches outlined above, mitigating overall autoimmune reactivity to the transplanted islets.

Conclusion

The engineering of a 3-D microenvironment for islet transplantation can provide a means to recapitulate the natural dynamic matrix environment of the islet niche. Moreover, the selection of an appropriate transplant site with desirable mechanical and biological properties can enhance the long-term success of the graft. Of note, the full potential of these 3-D biomimetic platforms may be in directing β -cell differentiation from stem cells, whereby spatial, bioactive cues can be provided to guide cell fate and facilitate appropriate cluster morphology. Overall, engineered biomimetic 3-D scaffolds have been shown to be versatile platforms for addressing many of the key challenges hindering the success of islet transplantation.

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HIGHLIGHTS

- Development of 3-D niches to support islet engraftment and retain function
- Incorporation of native ECM proteins minimize islet anoikis
- Islet grafts benefit from recent advancements in strategies to supplement oxygen
- Bioactive materials facilitate and accelerate vascularization for islet implants

Figure 1.

Various novel approaches implemented within islet transplants to improve overall function and efficacy include: (A) macroporous scaffold designed to provide mechanical protection and 3-D distribution of islet clusters; (B) co-encapsulation of islets with helper cells such as MSCs or ECs to promote revascularization and engraftment; (C) incorporation or tethering of growth factors to biomaterials to hasten and direct the revascularization process; (D) implementation of devices/materials to supplement oxygen; and (E) incorporation of ECM proteins to recapitulate native islet niche. (*Illustration courtesy of Jessica A. Weaver*)

Figure 2.

Incorporation of native cell binding sites to material framework can minimize anoikis, induced by islet isolation procedures, islet dissociation, and/or β -cell culture. (A) Scheme employed to synthesize thiolated fusion protein EphA5 and ephrinA5. (B) Incorporation of thiolated proteins within PEG hydrogels via thiol-acrylate photopolymerization. (C) Representative live/dead (green=live; red=dead) z-stack confocal images of cultured β -cells, embedded within increasing concentrations (indicated by top panel) of functionalized PEG hydrogels, illustrate benefits of functionalization on β -cell clustering, particularly after extended culture periods. [32••]

Figure 3.

In situ oxygen generation approaches to improve oxygen profiles within implants. (A) Schematic of immunisolating macrochamber device, containing an embedded oxygen tank, implanted within a rodent model to evaluate islet engraftment, and immunohistochemical staining of immobilized islets for insulin (bottom left) and glucagon (bottom right). [43•] (B)Top: Schematic and photograph of an oxygen generating material based on CaO₂ encapsulation in PDMS. Bottom: Representative live/dead (green=live; red=dead) z-stack confocal images of rat islets co-cultured without (left) or with (right) a single CaO₂-PDMS disk and incubated for 24 hrs under 0.05 mM oxygen. [44•]