Design of Bioartificial Pancreas with Functional Micro/Nano-Based Encapsulation of Islets

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Abstract: Type 1 diabetes mellitus (T1DM), a devastating health issue in all over the world, has been treated by successful transplantation of insulin secreting pancreatic islets. However, serious limitations such as the requirement of immunosuppressive drugs for recipient patients, side effects as a result of long-term use of drugs, and reduced functionality of islets at the transplantation site remain. Bioartificial pancreas that includes islets encapsulated within semi-permeable membrane has been considered as a promising approach to address these requirements. Many studies have focused on micro or nano-based islet immunoisolation systems and tested the efficacy of encapsulated islets using in vitro and in vivo platforms. In this review, we address current progress and obstacles for the development of a bioartificial pancreas using micro/nano-based systems for encapsulation of islets.

Keywords: Bioartificial pancreas, islet encapsulation, type 1 diabetes mellitus, immunoisolation, islet transplantation, micro/nano-based encapsulation.

INTRODUCTION

Diabetes mellitus is an overwhelming health problem in the worldwide with numerous patients and immense costs [1, 2]. Currently, number of diabetics is approximately 171 million and World Health Organization (WHO) estimates that this number will double by 2030 [3]. According to International Diabetes Federation, the cost of treatment of this disease has reached $465 billion per year, with an increased annual global incidence rate of ~3% [4, 5]. Long term complications such as renal, cardiovascular, neural, ophthalmic problems, extensive morbidity rate for patients, and cost associated with the treatment are motivations for many researchers to work for the treatment of diabetes mellitus [6]. Among the treatment options, insulin therapy and pancreas transplantation have been clinically used techniques, while islet transplantation has been still considered as an experimental approach. Insulin therapy provides exogenous insulin uptake for a fair blood glucose control [7], where islet transplantation aims to replace missing beta cell function. Islet transplantation has the potential to maintain normoglycemia without the requirement of an invasive surgery [8, 9], while pancreas transplantation eliminates exogenous insulin uptake for 80% of patients through an invasive operation [9, 10]. The basic advantage of islet transplantation over whole pancreas transplantation is the possibility to avoid immunosuppressive drug uptake, through an efficient design of immunoprotective coating of islets before transplantation [11, 12]. Coating also opens up the possibility of implantation of islets into alternative sites and transplantation with xenogenic cell sources to address the limited number of donors [13, 14]. Transplantation of islets is still not the best solution for T1DM due to several limitations, such as difficulty for finding pancreatic tissue donor, requirement for life long use and deleterious effects of immunosuppressive drugs on beta cells and the immune system of the host, post-operative complications, and high expense of the method [10, 15-18]. In this paper, recent progress for the successful development of a bioartificial pancreas based on micro/nano encapsulation strategies have been reviewed and novel immunoisolation approaches to move this technology forward are discussed in detail.

TREATMENT OPTIONS FOR DIABETES

One of the treatment approaches for diabetes is insulin therapy (Fig. 1) [19]. Insulin therapy is applied through multiple daily injections, where patients need three or more injections per day [20]. However, insulin therapy is not sufficient to provide normoglycemia since exogenous insulin cannot fully mimic endogenous insulin [19, 21]. It may also lead to insulin resistance and severe hypoglycemia [22, 23]. Day-to-day variability of insulin requirements, fluctuating absorption profiles of insulin, the daily burden of multiple insulin injections or diet adjustments based on finger stick blood glucose determinations complicate insulin therapy [9, 24]. In addition, patients must receive lifelong education about blood glucose to keep their blood glucose levels in the targeted range [20, 25]. New combinations of insulin analogues, introduction of insulin pumps and improved home blood glucose monitoring devices may relieve the drawbacks associated with insulin therapy [19, 21, 24, 26-29].

The other approach to control blood glucose levels is the use of mechanical insulin pumps which can be applied sub-
In this therapy, exogenous insulin is delivered into the body with an insulin pump instead of syringes or pens to mimic physiological insulin release [30, 31]. The mechanical pump provides advantages over multiple injections such as overnight glycemic control, ease of administration, reduced timing and concerns about the amounts of food intake, adjustable insulin delivery pattern and achievement of basal rate [20, 30, 32]. However, the drawbacks of pumps are associated with infection at the insertion site, ketoacidosis, unexpected insulin swings, and hypoglycemia [31, 33, 34]. Moreover, patients have to carry an external pump, which may not be very convenient. Even though there exists some implantable insulin pumps, there is a possibility of formation of undesirable coating of the catheter with fibrin clots [34].

Another strategy used to control blood glucose level involves pancreas transplantation [15, 35]. Pancreas transplantation provides long term insulin independence to patients who cannot maintain normoglycemia with insulin therapy or do not want to continue a tight diet and multiple injections [36]. The International Pancreas Transplant Registry reported 95% patient survival rate between 1996-2008 for the first year, where patient survival and graft function increased after 2011 [37, 38]. Pancreas transplantation is heavily invasive and carries the highest risk of surgical complications among transplanted solid organs due to the intense immunosuppression requirement, multiple vascular and enteric anastomoses [36, 37]. The surgical complication incidence associated with pancreas transplantation is over 20% and diabetes mellitus reoccurs in 17% of patients 5 years after transplantation [39].

Transplantation of insulin secreting islets to diabetic patients has also been developed to manage blood glucose level. The idea of transplantation of pancreatic islets was improved with Edmonton protocol for islet isolation from a guinea pig [40, 41]. Between 1960 and 1980, various clinical and animal trials of islet transplantation were performed [41-44]. Since then, the success of autograft islet transplantation increased and the purity of islets was improved [45-47]. This approach allowed transplantation of well characterized, viable islets with steroid free immunosuppression [48]; however, it was still a challenge to maintain long term insulin independence [48-52]. With the progress of new immunosuppression regimens and islet isolation protocol, one-year insulin independence rate was raised to 65% by 2012 [53-56].

**IMMUNOISOLATION OF ISLETS**

Immune rejection is still an unsolved problem of islet transplantation, although immunosuppressive agents continually evolve [57]. Hindering transplanted cells from the immune system is a must to keep transplanted cells viable and functional [58-61]. For this reason, immunoisolation is applied to coat or hide metabolically active cells within a selectively permeable membrane barrier [58, 59].

Common to various immunoisolation designs are a perm-selective membrane, an internal matrix and living cells [59]. These barriers constructed with either natural or synthetic materials differ in size over several orders of magnitude from small spheres, with a volume of $10^{-5}$ cm$^3$, to large extracorporeal tools with a net volume of 10 cm$^3$ [58]. Further, these immunoisolation systems are classified into two groups:
Vascular devices and nonvascular devices [58]. Vascular devices, which can interact with blood require the use of an anticoagulant agent to prevent thrombosis. Nonvascular devices in the form of spherical microcapsules or larger polymeric macrocapsules are designed for transplantation into body cavities [59]. Macrocapsules can be fabricated as sealed cylindrical hollow fibers, flat sheet, and planar devices with one order of magnitude below 1 mm to maximize bidirectional diffusion of nutrients and cellular therapeutics. Spherical microcapsules are made of synthetic or natural polymers such as sodium alginate, poly-L-lysine (PLL), agarose, poly(ethylene glycol) (PEG), chitosan, and multilayered glycol chitosan-alginate complexes [58]. Even though macrocapsules have better mechanical strength and chemical stability than microcapsules, a large volume of the macrocapsules hinders mass transport and may cause a decrease in viability of encapsulated cells [59]. The first immunobarrier membrane for islets was employed in 1977 by Chick et al. in the form of hollow tubes from a semi-permeable acrylic copolymer [62]. Later, perm-selective microcapsules were made of alginate, PLL and poly (ethylene imine) were preferred for islet immunoisolation to overcome aforementioned limitations of macrocapsules [57].

Host Response to Materials

Immune rejection is the main host reaction against cellular therapeutics such as allogenic, xenogenic or ex vivo modified autologous cells [63]. Insertion of a biomaterial into the body triggers a foreign body reaction which induces nonspecific adsorption of various proteins onto the surface of transplanted materials in native or denatured conformations, followed by migration of macrophages, leukocytes and platelets to the implant surface [64]. After activation of macrophages, biomaterial surface is exposed to cytokines, growth factors, proteolytic enzymes, and reactive oxygen and nitrogen intermediates secreted by macrophages. Leukocytes (neutrophils and monocytes) accumulate around the transplanted biomaterials during inflammation reaction. Granulation tissue grows via proliferation of fibroblasts and vascular endothelial cells around implant after the reaction with monocytes and macrophages. This new tissue consists of new small vessels and fibroblasts. Macrophages which adhere to the surface of the biomaterial change into foreign body giant cells with great number of nuclei. Final phase of immune response to biomaterials is the main fibrous capsule formation that surrounds implants and triggers severe complications [63-65].

Natural and Synthetic Materials for Islet Immunoisolation

Biomaterials have been exploited as medical implants for at least 2000 years, but most of the transplanted materials broke down due to biological issues such as infection and biological reaction to the materials [64]. The properties of biomaterials such as chemical, toxicological, physical and mechanical features should be appropriate for therapeutic purposes, human health, and device performance [59, 60, 65]. The immunoisolation barrier must be biocompatible and inert to eliminate material toxicity and to inhibit host response [66]. Immunoisolation barriers are designed semipermeable, since transplanted cells can only survive if the influx and efflux rate of desired molecules such as nutrients, oxygen, therapeutic products and cellular waste are sufficiently controlled [67]. Since the purpose of islet transplantation is to recover a patient’s insulin secreting ability, transplanted islets must allow for the transport of synthesized insulin into the body. This insulin secretion could be achieved through appropriate design of membrane pore size which will also be important to prevent the diffusion of components of the immune system (Fig. 2) [58, 59, 67, 68]. Furthermore, immunoisolation barrier must withstand mechanical and osmotic stress conditions in physiological environment [69]. In their natural environment, cells are covered within extracellular matrix (ECM) which contains extracellular proteins and polysaccharides [60]. The complicated interactions between islet cells and ECM contribute to healthy development, homeostasis, and regeneration after injury or stress [70]. It can be asserted that mimicking natural environment for transplanted islets is indispensable to produce long-term viable and functional islets. In order to mimic natural ECM, various approaches have been used such as extracted biological matrix, basement proteins, short biofunctional peptide fragments, glycosaminoglycans and cell-modulating factors to improve islet function [70]. Usually, materials that are used for encapsulation of islets are in the form of hydrogel due to large hydration capability of hydrogels and similarity of those gels to the native ECM. Natural and synthetic materials (Tables 1 and 2) in the form of cross-linked structures are exploited to provide immunoisolation barriers for islets [68].

![Immune Cells](image)

**Fig. (2).** Schematic illustration of islet immunoisolation.

Natural Materials

Alginate, anionic polysaccharide from seaweed, has been widely studied as immunoisolation barrier due to its biocompatibility [57, 60]. Alginate-based immunobarriers have been employed in clinical trials for the treatment of TIDM [71]. Molecules of this polysaccharide (chains of mannuronic acid (M) and guluronic acid (G)) show specific binding to multivalent cations such as calcium and magnesium that induce cross-linking of alginate [71]. These electrostatically crosslinked alginate-based immunoisolation coatings do not hinder cellular function of islets; however, there exists various concerns about the interaction of alginate with cells and its mechanical stability. For example, binding of alginate to a negatively charged cellular surface is restricted due to the...
Table 1. Natural materials used for the design of immunoisolation barrier.

<table>
<thead>
<tr>
<th>Material</th>
<th>Chemical structure</th>
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<td>Alginate</td>
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<tr>
<td>Agarose</td>
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<tr>
<td>Chitosan</td>
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<tr>
<td>Collagen</td>
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Negative charge of alginate [72]. Also alginate-polycation systems such as PLL, poly (ethylene imine), poly-L-ornithine (PLO), poly-D-lysine, chitosan and poly (methylene-co-guanidine) are employed to overcome limited mechanical stability issue [72]. Lim and Sun produced alginate-PLL immunobarrier with poly (ethylene imine) for islet transplantation into rats; however, alginate capsule resulted in foreign body reaction after 2-3 weeks [43]. Similarly, P. de Vos and his colleagues investigated physicochemical changes of alginate-polysine beads in vivo, where they observed that alginate-polysine bead was subjected to crucial physicochemical alteration, and that these changes activated immune system [73]. When alginate was used with collagen, the material showed significant potential as an immunoisolation barrier due to the minimized harm to islets during encapsulation and homogenous coatings around islet spheroids (Fig. 3) [74]. These coated islet spheroids sustained glucose level below 200 mg/dL for 4 weeks after implantation into the intraperitoneal cavity of mice [74].

Table 2. Synthetic materials used for the design of immunoisolation barrier.

<table>
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<tr>
<th>Material</th>
<th>Chemistry</th>
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<tbody>
<tr>
<td>PEG</td>
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<tr>
<td>Poly (N,N-dimethyl acrylamide)</td>
<td><img src="image" alt="Poly N,N-dimethyl acrylamide" /></td>
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<tr>
<td>Hydroxymethylated Polysulfone</td>
<td><img src="image" alt="Hydroxymethylated Polysulfone" /></td>
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<tr>
<td>Poly(vinyl alcohol) (PVA)</td>
<td><img src="image" alt="Poly(vinyl alcohol) (PVA)" /></td>
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<tr>
<td>Copolymer of D,L-lactide and glycolide (PLG)</td>
<td><img src="image" alt="Copolymer of D,L-lactide and glycolide (PLG)" /></td>
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<tr>
<td>Polyurethane</td>
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Agarose is another type of polysaccharide extracted from seaweed. Due to its thermosensitivity, this natural polymer has been employed for coating of islets within temperature ranges of 15-30°C [57]. Kobayashi et al. investigated immunocamouflage properties of agarose microcapsules of 100 to 400 µm in diameter (Fig. 4). The authors did not observe mononuclear cellular infiltration for more than 3 months, where efficient blood glucose could be achieved in diabetic mice that received agarose encapsulated islets [75]. However, agarose could not provide long-term immunoisolation property in physiological conditions, as was observed in another study [60]. Modification with polystyrene sulfonic acid, polybrene, carboxymethyl cellulose and combination with biological cues such as soluble domain of human complement receptor 1 were proposed as better alternatives to unmodified agarose [76, 77].
Chitin, a natural polymer, located in the shells of crustaceans and insects, is an appropriate material for biomedical applications due to its biocompatibility and easy handling. Chitin contains N-acetyl-glucosamine and N-glucosamine units, and when N-acetyl-glucosamine part becomes dominant in the structure, the polymer is named as chitosan. Chitosan is synthesized via deacetylation of chitin [57, 78]. Cross-linked chitosan has been extensively employed in diverse areas such as wound healing, plastic surgery, dental implants and islet encapsulation [60]. For example, Yang et al. encapsulated rat islets within chitosan microcapsules for immunoprotection of islets. Histological examinations demonstrated that grafted chitosan hydrogel coated islets at the renal space of mouse could secrete insulin, and that chitosan hydrogel coating could protect islets from immune cell attack for about 4 weeks (Fig. 5) [79].

Collagen is another commonly used natural polymer that is widely found in mammalian connective tissues [67]. It is extensively exploited in biomedical applications due to its biocompatibility, biodegradability and natural ability to attach cells [67]. Collagen in the form of crosslinked gel has been preferred for cell encapsulation, as crosslinked form of collagen can function as a cage to retain cells [80]. Although collagen gels demonstrate lower mechanical strength, it allows reinforcement with other materials and these properties makes it an attractive tool for various devices [81].

**Synthetic Materials**

PEG is an FDA approved and commonly used synthetic material with well-known biocompatibility and low-toxicity. This polymer has extensive use in biomaterials, biotechnology, and medicine, and has exceptional features such as its linearity, non-ionic structure and hydrophilicity [82, 83]. PEG has minimum surface energy in water and due to this minimum interfacial energy, protein adhesion on PEGylated surfaces could be significantly reduced and non-specific interactions between PEG and proteins could be eliminated [83]. Due to these unique properties, PEG has also been considered for coating of insulin secreting islets and for improving the functionality of islets [84]. In one such studies, Kizilel et al. demonstrated that an insulinotropic ligand, glucagon-like peptide-1 (GLP-1), conjugated to PEG polymer could enhance viability and functionality of islets when covalently bound to pancreatic islets (Fig. 6) [84]. In addition, Lee et al. applied multiple layers of PEG coating of islets and observed that immune rejection could be delayed for 100 days in diabetic patients [85]. Long-term stability of PEG-based barrier could be achieved through synthesis of cross-linked PEG hydrogels around cells and this strategy has been used in previous studies. Photopolymerized PEG hydrogels around pancreatic islets have been observed to provide immunoprotection by limiting diffusion of immune system components through the hydrogel without compromising the function of encapsulated islets [86]. For instance, Cruise et al. coated pancreatic islets within PEG hydrogel using interfacial photopolymerization and showed that PEG hydrogel coating works as an effective immunoisolation barrier to
The histological studies of chitosan hydrogel encapsulated islets. The histologic sections of islets/hydrogel group show that (a) the islets were transplanted at the renal subcapsule space of mouse (The sections were stained with hematoxylin and eosin, 40×). (b) Immunohistochemical staining shows that the islets had positive insulin staining (red color, immunostain of insulin, 100×). Sections stained with antibody specific to immune cells show there was no immune cell infiltration or accumulation. (c) Negative of CD3+ T-cell lineages (40×). (d) Nor of CD68+ monocyte/macrophages (100×). (Adapted with permission from Yang, Qi et al. 2010. Copyright 2010, Elsevier).

exclude large molecules or immune cells, while allowing islets to respond to dynamic or static changes in glucose concentration by producing insulin [87, 88]. However, encapsulation of islets within nonfunctionalized PEG hydrogel is not sufficient for long-term islet viability and functionality. Hence, studies considered incorporating insulinotropic peptides such as GLP-1 and islet basal membrane derived proteins such as collagen and laminin into the gel network to enhance the function and survival of islets [89, 90].

Cross-linked poly (vinyl alcohol) (PVA) foam has also been considered as a synthetic matrix for encapsulation of islets or other cells due to low protein binding tendency, high water content and elasticity [60, 91]. Bulk encapsulation of islets in PVA hydrogel sustained normoglycemia in diabetic rats for 12 days [92]. Teramura et al. developed multilayers of PVA coating of islets through maleimide-PEG-lipid anchoring on cell membrane surface. The authors obtained ultra-thin layers of PVA around islets, and still maintained islet survival and function [93]. Another example of PVA encapsulated islets was developed by Qi et al., where the authors macroencapsulated islets within PVA and observed successful allotransplantation of PVA encapsulated islets without administration of immunosuppressive drugs. It was also reported that insulin positive islets were still present during week 4, at post transplantation and that the body weight loss was minimized in the recipients of PVA coated islet group [91].

Synthetic biodegradable copolymer of D,L-lactide and glycolide (PLG) has been considered as alternative polymer to generate 3D coating around islets [94]. This FDA approved polymer can be designed to degrade with time-ranging from few weeks up to one year, where scaffolds of PLG can be considered to function as a platform for extra-hepatic islet cell transplantation. To test this hypothesis, Blomeier et al. used PLG microporous scaffold to reduce islet loss at the transplantation site due to limited blood flow. The authors observed that those islet implants were revascularized due to degradable PLG scaffold, and were found as insulin positive for 2-4 weeks [94]. Moreover, Gibly et al. designed PLG microporous scaffolds to improve cell infiltration and to provide revascularization of islets with minimum amount immune response [95].

Poly(N,N-dimethyl acrylamide) (PNNDA) synthetic hydrogels have also been considered as potential scaffolds in tissue engineering due to desirable mechanical stability, preservation of integrity during transplantation, and permeability to nutrients and oxygen with controllable pore size [96, 97]. These attractive properties have been found useful for islets macroencapsulated within PNNDA, where they remained viable and functional for about 45 days [97]. Further, modification of PNNDA with polydimethylsiloxane (PDMS) was considered to protect islets from immune attack without any prevascularization strategy or immunosuppressive therapy, where normoglycemia up to 3 weeks in pancreatectomized canines was observed [98].

Polysulfonate is another synthetic polymer that has been considered for islet encapsulation. Hydroxymethylated polysulfonate capillaries were designed by Lembert et al. for vascular tissue formation [99]. It was observed that the capillaries provided vascular growth without interfering insulin release kinetics [11, 57, 100, 101].
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Fig. (6). Differential interference contrast microscopy images of (a) untreated islets and (b) islets that were coated with biotin-PEG-NHS/SA/biotin-PEG-GLP-1. Scale bar=200 μm. (Adapted with permission from Kizilel et al., 2010. Copyright © 2010, Mary Ann Liebert, Inc).

Polyurethane synthetic polymer has high strength, optically transparent and nonporous structure [102]. Dense polyurethane membranes were exploited as potential immunoisolation barrier for islets. However, significant success was not observed due to retention of molecules within the polymer. Modification of polyurethane through polytetramethylene glycol and butanediol improved biocompatibility, permeability to glucose and insulin, while islets were protected against the attack of immune system [103].

Strategies Used for Islet Encapsulation

The idea of encapsulation of cells within natural or synthetic 3D scaffolds involves enclosure of viable cells or tissues within semi-permeable membranes [104]. These scaffold networks should allow for the diffusion of nutrients, oxygen and cellular waste while preventing the interaction with immune system constituents [105-107]. Encapsulation has been not only been considered for immunoisolation purposes, but can also be used to enhance cell viability and function using biomaterials at various geometries. However, for the condition of islet encapsulation, an optimal balance has to be adjusted among distinct membrane features to promote islet cell viability with desirable insulin secretion function in response to glucose [104]. Natural or synthetic polymers discussed above have been considered for micro-, macro-, or nano-encapsulation of pancreatic islets (Fig. 7). In addition, cellular coating has been considered as an approach to replace polymeric coating of islets. Below, these strategies have been discussed in detail.

Fig. (7). Islet encapsulation strategies for immunoisolation.

Macroencapsulation involves placing of groups of islets within a selectively permeable membrane which can be designed as an intravascular or extravascular macrocapsule [105, 107, 108]. Intravascular macrocapsules refer to microporous or nanoporous perifusion chamber that is directly connected to a vein, where extravascular macrocapsules are prepared as a tube or sphere to function as a flow hollow. The inner diameter of the hollow alters from 0.5 mm to 1.5 mm, where the axial length ranges between 1 and 10 cm [86]. Qi et al. used PVA macrocapsules for transplantation of rat islets into the peritoneal cavity of C57BL/6 mouse. Although there has been decrease in insulin secretion capacity after freeze-thawing, islets encapsulated within PVA hydrogels supplemented with Euro-Collins solution (a cryoprotective solution) demonstrated comparable functionality with free islets and hyperglycemia was reversed in diabetic mice within 4 weeks [106]. The design of a bioartificial pancreas through the use of macroencapsulation approach may provide better durability than those prepared via microencapsulation. Since macroencapsulated islets can be implanted within peritoneal cavity or subcutaneous site, macroencapsu-
lation allows for the recovery of the graft with minimal surgical risk [86, 106, 109, 110]. The disadvantage of this approach is related to the large sizes of these constructs, where thick hollow platform and large capsule diameter limit the diffusion of physiologically important molecules, resulting in reduced viability and delayed insulin secretion in response to glucose [105].

Microencapsulation refers to the encapsulation of individual or few islets in a spherical capsule, where the diameter ranges between 0.02-1.5 mm [100, 104]. Various techniques have employed microencapsulation of islets using photopolymerization, double emulsion, micro-machined nanoporous microsystems and electrified coaxial liquid jets techniques [100, 104]. Soon after the first report of islet microencapsulation by Lim and Sum in 1980, scientists recognized the possibility of application of this strategy for the treatment of TIDM [111]. In the following years, various natural and synthetic materials were considered to design bioartificial pancreas in vitro or in vivo. Those studies mainly focused on elimination of immune suppression requirement with islet transplantation, and improving function and viability through vascularization of constructs [112-116]. Microencapsulation approach has various advantages compared to the other immunoisolation techniques. It results in the formation of capsules with bigger surface to volume ratio that allows relatively high diffusion rates of oxygen, insulin, nutrients and waste simple transplantation, and retrieval of capsules [12]. The diameter of microcapsules should range between 300-400 µm in order to obtain desirable permeability property [100, 117, 118]. The microstructures have been designed to improve insulin secretion capability and to limit the diffusion of immune system components. For example, insulinotropic agents such as hypoglycemic drugs or GLP-1 have been used within the capsule to improve insulin secretion functionality of encapsulated islets [96]. In addition, high oxygenation approach was exploited via hemoglobin (Hb) to promote islet viability and functionality. Kim et al. designed a rechargeable bioartificial pancreas system that included thermoreversible polymeric ECM as an immunoisolation barrier with insulinotropic agents and oxygen transporting molecules [96]. Microcapsules with Hb could improve insulin secretion and viability of islets as well as resulted in euglycemia for about 8 weeks in diabetic mice. Recently, Ma et al. proposed alginate-based microencapsulated islets with core-shell structure using droplet generator under electrostatic force [119]. Fig. (8) demonstrates that uniform core-shell microcapsules of islets could be obtained with this approach, and that these islets provided glycemic control for about 80 days after transplantation into diabetic mice [119].

The main limitation of macro and microencapsulation strategies is related to the total size of the tissue to be implanted to cure a diabetic patient. In order to reduce total graft volume and to improve glucose response time, nano-thin coating strategies have been developed [57]. Krol et al. employed a multilayered nano-capsule for islet encapsulation through layer-by-layer self-assembly of polycations and polyanions on islet surface [120]. Even though it is possible to minimize the coating thicknesses through various microencapsulation strategies, the total volume of the encapsulated islets to be transplanted limits transplantation into the clinically preferred sites such as portal vein of the liver. To address this limitation of volume increase, Wilson et al. encapsulated individual islets within nano-thin membrane via layer-by-layer self-assembly of PLLg-PEG-biotin (PPB) and streptavidin (SA) (Fig. 9). The nano-thin coating did not compromise the viability, where islets in a multilayered nano-thin coating had similar survival rate and function compared to untreated islets [121].

Coating of islets within living cells have also been considered for binding of specific type of cells onto islet surface. Teramura et al. used cellular based coating approach through

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Fig. (8). Alginate-based microencapsulated islets: Comparison of islets encapsulated in (a) regular capsules and (b-e) core–shell capsules. (c-e) 3D reconstructed confocal fluorescent images of islets encapsulated in core–shell capsules. The islets were stained blue, while the shell was labeled green. Scale bar=500 µm for a-b and e. Scale bar= 100 µm for c-d (Adapted with permission from Ma, Chiu et al. 2013. Copyright 2013 John Wiley and Sons).
such as oxygen gene hypoxia (DFO) to antiaototic gene secretion. Islets may be treated with d mammals can be mimicked. H molecules and cytokines including vascular endothelial may i survival and function induced injury of islets should be addressed to improve islet should be rapidly revascularized. Hypoxia/reoxygenation is not desi causes severe hypoxia and nutrient deprivation. Since h the recipient patient could be considered for clinical islet transplantation, where T regulatory cells of modulation may open up the possibility of using T regulatory cells foring insulin secr cell mediated reaction could be achieved without compr cells could completely cover islet surfaces after 1 in day cu streptavidin interaction to coat islets within CD4 pancreas islets has been utilized to promote local immuno protection on islet surfaces. Marek et al. used biotin-streptavidin interaction to coat islets within CD4+ CD25 high CD127 T regulatory cells. Fig. (11) shows that T regulatory cells could completely cover islet surfaces after 1 in day culture. It was observed that effective protection from effector T cell mediated reaction could be achieved without compromising insulin secretion function in response to high glucose [124]. The idea of using T regulatory cells for islet encapsulation may open up the possibility of using T regulatory cells for clinical islet transplantation, where T regulatory cells of the recipient patient could be considered for in vitro expansion and used to coat allogeneic pancreatic islets.

After islet isolation, islets become avascular which causes severe hypoxia and nutrient deprivation. Since hypoxia is not desirable for cellular function and survival, islets should be rapidly revascularized. Hypoxia/reoxygenation induced injury of islets should be addressed to improve islet survival and function [125-127]. Mesenchymal stem cells may be utilized with islet grafts against hypoxia since they may inhibit cell growth and differentiation through signaling molecules and cytokines including vascular endothelial growth factor, hepatocyte growth factor, nerve growth factor and leukemia inhibitory factor [126]. Alternatively, a homeostatic mechanism against reduced oxygen tension in mamals can be mimicked. Hypoxia inducible factors such as HIF-1α combined with aryl hydrocarbon receptor nuclear translocator (ARNT) regulates cellular response against hypoxia and increases vascular endothelial growth factor and antiaapoptotic gene secretion. Islets may be treated with deferoxamine (DFO) to increase HIF-1α production against hypoxia [128]. Other strategies have been also considered such as oxygen generating polydimethylsiloxane encapsulated solid calcium peroxide [129], or perfluorocarbon emulsions oxygen carriers in order to address islet hypoxia.

CHALLENGES ASSOCIATED WITH ISLET TRANSPLANTATION

Survival rate of graft and patients, life quality and maintenance of normal metabolic state in the long run determine the success of islet transplantation [35]. Transplanted islets are expected to respond to host glucose levels by secreting insulin similar to normal pancreatic islets [130]. American Diabetes Association (ADA) defines islet transplantation as an experimental operation which is only allowed in Food and Drug Administration (FDA) controlled research centers or laboratories [6]. Among the challenges of islet transplantation are loss of islets during isolation [29], loss of islet viability during or after transplantation, limited supply of islets [131], exhaustion of already limited islet mass and deleterious effects of immunosuppressive drugs [15]. In their native environment, islets of Langerhans are highly vascularized with a dense glomerular-like capillary network which enables islet communication and intra islet blood flow for normal islet function [132]. Islets contact with ECM that evokes complex cellular processes [89], and during islet isolation process, surrounding microvasculature [74] and islet-matrix interactions are destroyed. Due to these reasons, survival and function of islets and hence success of islet transplantation procedures decline [89]. In order to increase the success of islet transplantation, islet matrix interactions might be reestablished after isolation, or immunogenicity might be reduced through in vitro culturing of islets before transplantation to ameliorate islet transplantation [89, 132-135].

Source of Islets

An adult human pancreas contains 0.3-1.5 x 10⁶ islets with 2x10⁶ beta cells [136], and a successful isolation may yield about 400,000 islets at most since only 30-50% of the islets can be isolated, where about 65% of those are viable [127]. However a standard transplant recipient requires 0.35-1.0 million islets since patients require around 15000 islet equivalents (IE) per kilogram of patient weight to become insulin independent. Additionally, these islets should have standard diameter of 150μm [47, 48, 50]. Therefore, there is a great gap between available source of islets and patients who need islet transplantation. This limitation highlights the
Fig. (10). HEK293 cells encapsulated islets: Phase-contrast microscopy of HEK293 cell-immobilized islets in culture at 0-5 days. HEK293 cells were immobilized on the surface of the islets and cultured on a non-treated dish in Medium 199 at 37°C. Arrows indicate immobilized HEK293 cells. Scale bar=200 µm for a-c and 100 µm for d. (Adapted with permission from Teramura and Iwata 2009. Copyright 2009, Elsevier).

Fig. (11). Treg-encapsulated islets: Tregs are effectively attached to the islets. Confocal microscopy imaging after 1-day culture. (a) Three dimensional view of a human islet coated with T cells (nuclei stained with Hoechst shown in blue) reconstructed from a stack of 18 optical images with 5-µm increment. (b) Alternative viewing angle of the islet shown in A. (c) Bright field image of the human islet. (d), Cutting plane view reconstructed from T cell coordinates exposing the coating layer of T cells surrounding the unstained islet cells. (Adapted with permission from Marek, et al. 2011. Copyright 2011, Lippincott Williams & Wilkins, Inc.).
urgent need of cell sources to bring this technology closer to clinical use [136]. Islet sources from humans can be obtained from the patient himself, which is called autograft and hence, transplantation does not evoke an inflammatory reaction [132, 137]. Until 2000, 222 islet auto-transplantations were performed in the worldwide after pancreatectomy and nearly half of the patients gained insulin independence [138, 139].

Allograft islet transplantation requires islets from different individuals of the same species such as pancreas of cadaveric donors [131]. Since brain-dead donors have up-regulated proinflammatory cytokines, allograft transplantation confronts immune response of the recipient [140]. Currently 65% of the diabetic patients have become insulin independent within the first year of islet allotransplantation and 5 year insulin independence rates range between 60-70% [53]. Xenotransplantation refers to primary islets or other insulin secreting tissue sources from other organisms such as porcine, rat, mouse, bovine, rabbit, trout and fish brockman bodies [35, 131, 141]. Porcine is the main alternative donor species, since human and pig insulin have structural similarity [131]. Also, pig islets efficiently proliferate in vivo and in vitro [142] and can be easily genetically manipulated compared to that of islets from primates [143]. However, xenograft islets from pig evoke hyperacute immune rejection due to the presence of nonhuman moiety, α-1,3 galactosyltransferase, on islet surface [131] and the rejection is primarily mediated by CD4⁺ T cells and by a minor effect of CD8⁺ T cells in the presence of xenoreactive antibodies through the indirect pathway of antigen presentation [144, 145]. In addition, porcine endogenous retroviruses may trigger a cross-species infection in recipient patients [131].

Besides the possibilities of obtaining islets from autogenic, allogenic or xenogenic sources, insulin secreting cells can be obtained by genetic engineering approaches [66]. These cells or islet-like clusters easily grow under sterile conditions to high masses while allograft islets from cadaver donors hardly proliferate. This expandable and functional cell source addresses the insufficient islet problem [146, 147]. These cell sources include tumors and transformed cell lines [146], and beta-like cells derived from induced pluripotent stem cells that were shown to be effective to reverse diabetes in vivo [148, 149]. These somatic cells such as liver cells and K cells [150-152], pancreatic ductal cells [146] and fibroblasts [153] can also be reprogrammed into insulin producing cells via insulin gene delivery [150, 151]. In addition, through the use of gene therapy techniques, animal donors which lack glycosylation property can be generated to reduce xenograft rejection [143, 147, 154].

The limitation about obtaining sufficient islet source could also be addressed through stem cells which have self-renewal and differentiation capacity [131, 155]. Embryonic stem cells, embryonic germ cells, embryonic carcinoma cells, mesenchymal stem cells, induced pluripotent cells from somatic cells [148] and adult pancreas stem cells [156-158] can be utilized for islet transplantation [146, 155, 159]. These cells can be reprogrammed into beta cells if appropriate conditions for growth and differentiation are satisfied, and they can be used to normalize blood glucose levels [146, 160-165]. Adult stem cells and induced pluripotent stem cells do not evoke alloimmune rejection, but embryonic stem cells do [148, 166]. Additionally, mesenchymal stem cells enhance graft survival and function when they are physically co-transplanted with islets, since they reduce required beta cell mass and promote tissue vascularization [167-173], provide anti-inflammatory and immunomodulatory properties and secrete antiapoptotic paracrine factors to enhance islet viability [126, 174-176]. However, the level and induction of insulin secretion from stem cells should be quantified [177]. Risk of mutagenesis due to vectors used for reprogramming and possibility of tumorigenic properties should also be investigated in detail before stem cells can be treated as a treatment option for diabetes [152, 156, 178].

Primary beta cells as major endocrine cells to replace islets of Langerhans have been considered in cell therapy for the treatment of diabetes [179]. However, primary beta cell donation is limited and these cells have limited proliferative capacity [141, 180]. Immortalized beta cell lines may address this problem, as they are uniform, can easily proliferate and secrete insulin [141, 179, 180]. Beta cell lines may be produced from pluripotent stem cells, embryonic germ cells, embryonic carcinoma cells, bone marrow stem cells or spermatogonial stem cells via genetic and cellular engineering techniques [136, 146, 152]. RNIm5F, MIN6 and HIT are some of the immortal pancreatic beta cell lines from transgenic mouse that are under experimental trials for replacement of islets to cure TIDM [179]. However, beta cell lines which contain all properties of primary beta cells have not been produced yet [179, 180]. Also, due to the carcinogenic history, beta cell lines cannot be transplanted to diabetic patients clinically. They are usually examined to understand insulin secretion and growth properties of primary beta cells according to matrix-cell interactions and cell-cell interactions [90, 136, 180].

**Site of Transplantation**

In order to optimize islet engraftment, function and to reduce required islet mass for transplantation, researchers seek alternative sites for transplantation. An optimum transplantation site must provide sufficient blood supply, high oxygen and must have angiogenesis capacity, as beta cells cannot properly function without sufficient vasculature [181, 182]. The optimum site of transplantation must also minimize surgical complications and must require the lowest mass of islets [183]. This site must further provide portal delivery of insulin and be easily accessed to allow minimally invasive operations [184]. Spleen, epiploic pouch, peritoneal cavity, testis, thymus, kidney capsule, brain, subcutaneous tissue, pancreas, eye, lung, epididymal fat and vascularized small intestinal segments have all been studied for transplantation of islets [181, 185-189]. These sites have their own disadvantages and advantages. For instance, grafts within the lung presented poor performance [187], where liver, spleen, kidney capsule, striated muscle are highly vascularized sites, and subcutaneous and intramuscular sites are sparsely vascularized [132, 185]. Renal subcapsular space provides a fast vascular engraftment and desirable growth conditions for islets [190, 191]. However, this site is small and exocrine contamination destroys islets [182, 184]. Epididymal fat pad yields similar glycemic control efficacy to intraportal islet transplantation [188]. Testis, thymus, brain and eye are im-
mune-privileged, and the iris of the eye is also highly vascularized with a rich autonomic nerve network and provides easy imaging [181, 186]. In vascularized small intestinal segments, vascular endothelial growth factor, hepatocyte growth factor, fibroblast growth factor-2 and transforming growth factor-β are expressed which accelerate islet graft revascularization [189]. An optimal site for islet transplantation has not yet been reported; however, currently, intraportal islet transplantation is the predominant method, because insulin is intrinsically metabolized in liver [42, 192, 193]. Between 1990 and 2000, 92% of the clinical islet transplantation cases were performed into the liver via portal vein injection or infusion [55]. Liver provides oxygen plus nutrient rich environment, hepatic uptake of nutrients and hormones, simple and cheap access to the transplantation site and portal insulin drainage with a normal rate. Therefore, it reduces hyperinsulinemia risk and insulin secretion delay [35, 66, 183, 192, 194]. However, liver has many disadvantages such as insufficient islet vascularization and the risk of various complications [132, 183, 194]. Intense immune rejection, instant blood mediated inflammatory reaction, immunosuppressive drug and toxin accumulation may cause graft loss [46, 94, 132, 182, 195].

Intraperitoneal site has a large volume to accommodate encapsulated islets; however, it provides relatively low oxygen and blood supply to islets, and contains many macrophages. Also, the peritoneal cavity retards insulin release, as it is not a regular insulin delivery route [69, 194]. Many studies have conflict about the efficacy of intraperitoneal transplantation of islets to achieve insulin independence for patients [94, 196, 197]. Pancreas is the intrinsic location of native islets. The transplanted islets maintain their functionality and anatomy better in the pancreas than in the liver through intraportal transplantation [198]. This site has an insulin delivery to the portal vein and a high angiogenesis potential [198, 199]. The omentum pouch provides portal venous drainage, easy access for clinical follow-up, high vascular density and neoangiogenesis capacity [192]. However, omental islet transplantation has not been clinically studied yet and requires a higher amount of islet mass than intraportal and splenic transplantation [183, 192, 200]. Subcutaneous space is easily accessible for biopsy, guarantees maximum patient safety [201-203]. However, poor vascularization and low oxygen concentration in this site is the major challenge [185]. In order to address this issue, subcutaneous tissue or islets are prevascularized, transplanted with fibroblasts [201-205].

**Immunosuppressive Drugs**

When an allograft or xenograft is transplanted into a host, genetic inconsistency initiates numerous cellular, molecular and humoral immunological events. These events are induced by T and B cells, macrophages, and dendritic cells to reject foreign tissue [131]. Nonspecific inflammatory reactions respond to foreign tissue first; immune mediated destruction occurs later [35] (Fig. 12a). Especially, intraportally transplanted islets experience instant blood mediated inflammatory response (IBMIR). IBMIR involves complement and coagulation cascade activation and neutrophil infiltration [154]. It is a thrombotic reaction caused by direct contact of islets with ABO-compatible blood. In this process, activated platelets rapidly bind to the surface of the islets and a clot is formed around the islets that initiates thrombotic and complement activation cascades. Leukocytes infiltrate and islets suffer morphological defects [206, 207] (Fig. 12b). Along with these inflammatory reactions, T cells and other immune cell types are activated to evoke a cytokine-coordinated rejection. In allotransplantation, the rejection occurs through a direct pathway. When T cells recognize the represented antigens by donor MHC class II cells, CD4+ helper T1 cells are generated. These cells produce cytokines which expand cytotoxic CD8+ T cells. CD8+ T cells are called primary effector cells against allogenic cells (Fig. 12c). In xenotransplantation, rejection occurs through antigen-antibody reaction pathway and in this pathway, professional antigen presenting cells of the host represents donor antigens [131] (Fig. 12d).

In order to avoid immune rejection and autoimmune islet destruction, immunosuppressive drug uptake is crucial after pancreas and islet transplantation [19, 131]. Between 1999 and 2003, 80% of immunosuppression regimens were composed of IL2R antagonists. Therefore, recipients predominantly took calcineurin and IMPDH inhibitor combinations. Later, until 2009, usually a combination of calcineurin inhibitor and mTOR inhibitor was preferred after islet transplantation. Between 2007 and 2009, agents which induce T cell depletion regardless of the presence of TNF antagonists supplemented the immunosuppressive regimens [53]. Although immunosuppressive drugs inhibit acute rejection, they have many detrimental side effects such as increased malignancy elevated levels of reactive oxygen species in the host, cardiovascular problems, nephro- and neuro-toxicity, renal dysfunction, allergies, dermatologic diseases, inhibition of wound healing, mouth ulcers and alteration of the menstrual cycle in women [15, 19, 63, 131, 208-214]. During immunosuppressive drug therapy, a balance between toxicity and efficacy should be established [48, 66, 130, 181, 206]. Therefore, a personal dosage and schedule arrangement covering optimum timing and best fitting drug option is required. This procedure is very time consuming and difficult, as it depends on trial and error procedure [208, 215]. Immunosuppressive drugs may also have deleterious effects on beta cell function and structure while decreasing insulin gene expression and causing glucose mechanism abnormalities [216-220]. They can reduce host’s ability to fight disease by impairing immune system. Moreover, in spite of all envisaged risks, immunosuppressive agents may fail to prevent allo- and auto- immunity against transplanted islets. Therefore, new techniques to prevent immunosuppressive drug uptake are necessary. Hence, immunoisolation strategies have emerged to hinder allogenic and xenogenic islet grafts from host antibodies [15, 66, 136, 221]. The basic drawbacks of immunoisolation techniques are mass transport limitations and inflammatory responses to device materials [66].

**CONCLUSION**

T1DM is a common health problem affecting many people worldwide. To address this problem, many treatment options are developed such as insulin therapy, pancreas or islet transplantation. The treatment techniques are continuously improved to obtain the best method, which provides the most strict glycemic control. There is not an established
Fig. (12). Immune reaction against transplanted islets. 

**a) Islet Graft Rejection:** Upon contact of islets with ABO-incompatible blood, coagulation cascade (blue arrow) and complement cascade (pink arrow) starts. As a result of complement cascade, leukocytes are recruited to the region to perform allograft rejection via donor APC recognition (green circle) or xenograft rejection via host/donor APC recognition pathways (pink circle). 

**b) Instant Blood Mediated Inflammation Reaction:** IBMIR is a thrombotic reaction caused by direct contact of islets with ABO-incompatible blood. It involves complement and coagulation cascade activation and neutrophil infiltration. In coagulation cascade, the activated platelets rapidly bind to the surface of the islets resulting in clot formation around the islets. 

**c) Allograft Rejection:** Through direct pathway, as T cells recognize those represented antigens by donor MHC class II cells, CD4+ helper Th1 cells are developed which produce cytokines for expansion of cytotoxic CD8+ T cells as primary effector cells against allogenic cells. 

**d) Xenograft Rejection:** Light region (Direct Pathway): Donor antigen presenting cells are stimulated and host compatibility complex molecules (class II) are presented to T cells. MHCs displayed on donor xenotransplants may not effectively interact with host T-cells and may not mediate direct pathway. Dark Region (Indirect Pathway): Antigen-antibody reaction pathway, the antigens of the donor tissue are represented by the host professional antigen presenting cells which are macrophages and dendritic cells. Upon interaction of donor antigen representing cells and host T cells, CD4+ helper Th1 cells are developed which produce cytokines for expansion of cytotoxic CD8+ T cells as primary effector cells against xenogenic cells.
method or chemical regimen which completely cures diabetes mellitus; however, islet transplantation stands out as a novel and has potential to be improved through immunosolation options which hinder islets from host immune rejection, possibility to exploit various sources of islets and various transplantation sites.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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Design of Bioartificial Pancreas with Functional Micro/Nano-Based Encapsulation

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