Minireview *Highlight article*

Islet cell encapsulation – Application in diabetes treatment

Amoge Opara[1], Alec Jost[2], Sam Dagogo-Jack[3] and Emmanuel C Opara[1,2,4]

[1]Diabetes Section, Biologics Delivery Technologies, Reno, NV 89502, USA; [2]Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, Winston-Salem, NC 27157, USA; [3]Division of Endocrinology, Diabetes & Metabolism, University of Tennessee Health Science Center, Memphis, TN 38103, USA; [4]Virginia Tech-Wake Forest School of Biomedical Engineering and Sciences (SBES), Wake Forest School of Medicine, Winston-Salem, NC 27157, USA
Corresponding author: Emmanuel C Opara. Email: eopara@wakehealth.edu

Impact statement

It is generally believed that islet transplantation, which is a cell replacement therapy for type 1 diabetes, has the potential to cure the disease. However, two main barriers have impeded the promise of this treatment option for patients. These barriers are the acute shortage of human islets and the need to use toxic immunosuppressive drugs to prevent graft rejection. The concept of islet cell encapsulation emerged in 1980 as a strategy to overcome the two barriers and have now advanced to clinical trials. Still, the full potential of this technology remains to be realized. This minireview examines the various issues that have impeded progress and outlines new strategies to overcome the obstacles and enhance clinical translation.

Abstract

In this minireview, we briefly outline the hallmarks of diabetes, the distinction between type 1 and type 2 diabetes, the global incidence of diabetes, and its associated comorbidities. The main goal of the review is to highlight the great potential of encapsulated pancreatic islet transplantation to provide a cure for type 1 diabetes. Following a short overview of the different approaches to islet encapsulation, we provide a summary of the merits and demerits of each approach of the encapsulation technology. We then discuss various attempts to clinical translation with each model of encapsulation as well as the factors that have mitigated the full clinical realization of the promise of the encapsulation technology, the progress that has been made and the challenges that remain to be overcome. In particular, we pay significant attention to the emerging strategies to overcome these challenges. We believe that these strategies to enhance the performance of the encapsulated islet constructs discussed herein provide good platforms for additional work to achieve successful clinical translation of the encapsulated islet technology.

Keywords: Diabetes, islets, encapsulation, insulin delivery, bioengineering

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Introduction

Diabetes mellitus is a chronic metabolic disorder caused by either a relative or absolute failure of beta cells, leading to insulin deficiency. Insulin, secreted by beta cells in the pancreatic islets of Langerhans, is a key mammalian glucoregulatory hormone. Diabetes is diagnosed when fasting blood glucose concentration is >7.0 mmol/L (126 mg/dL), or random blood glucose concentration is >11.1 mmol/L (200 mg/dL) with symptoms. Other methods for diagnosing diabetes include a 2-h plasma glucose level of >11.1 mmol/L (200 mg/dL) during a 75-g oral glucose tolerance tests and hemoglobin A1c levels of >6.5%.[1]

Diabetes mellitus is commonly classified as type 1 diabetes mellitus (T1DM) or type 2 diabetes mellitus (T2DM). T1DM is characterized by severe insulin deficiency usually caused by immune-mediated destruction of beta cells, while T2DM results from insulin resistance and relative insulin insufficiency.[1] The etiology of both T1DM and T2DM involves an interplay of genetic and environmental factors with genetics playing a greater role in T2DM.[2] However, absolute or relative insulin deficiency is a cardinal feature of diabetes. Indeed, insulin secretion in amounts commensurate with insulin demand guarantees freedom from diabetes.[3]

Of note is the increasing prevalence of diabetes mellitus across all age groups.[4] The reasons for the increasing incidence of type 1 diabetes are unclear, whereas the rising incidence of type 2 is caused by rising urbanization, particularly in developing countries.[5] It has been suggested that one type of immune cell, the B lymphocyte, may play a role, but it is presently unknown what precipitating factors may enhance B lymphocyte activity leading to T1DM. It had

been shown that B cell depletion in new onset T1DM patients in the first Phase II trial of B cell depletion resulted in slowing the destruction of insulin-producing pancreatic beta cells. However, the mechanism for the beneficial effects of lymphocyte depletion remains to be determined.[6] Global incidence of diabetes is over 382 million with approximately 10% being T1DM.[7] T1DM is a chronic autoimmune disorder that can present acutely with life-threatening diabetic ketoacidosis (DKA). Long-term complications of uncontrolled T1DM include blindness from retinopathy, chronic kidney disease, and amputation from the combined effects of neuropathy and peripheral vascular disease. Diabetes also is the leading cause of macrovascular complications, such as cardiovascular disease and stroke, and a major driver of healthcare expenditure.[8]

There is compelling evidence from landmark clinical trials that the achievement and maintenance of optimal glycemic control prevents the development of long-term complications of diabetes. Due to the underlying severe or absolute insulinopenia, lifelong insulin replacement is the standard of care for controlling blood glucose levels in people with T1DM. Currently, exogenous insulin therapy is the predominant option for insulin replacement. Alternative approaches, based on cell-based therapies, include transplantation of whole pancreas or isolated islet cells and the emerging application of induced pluripotent stem cell technology. This review focuses on T1DM treatment by encapsulated islet transplant.

Historical perspectives

Islet isolation from the pancreas had been attempted earlier with poor islet yield. In 1967, a method of islet isolation from rat pancreas was reported by Paul Lacy and his team at Washington University in St. Louis MO.[9] The Lacy method involves injecting the digestive enzyme, collagenase, into the pancreatic duct before mincing the pancreas and digesting the tissue. That approach generated an improved islet yield and also demonstrated evidence of ability to isolate intact metabolically active islet cells. The Washington University group led by Dave Scharp subsequently isolated and purified human islets and successfully implanted them in the liver through the portal vein of a T1DM patient in the first clinical trial that resulted in no exogenous insulin requirement for 22 days.[10] Various modifications have been made on this method of islets isolation over the years with modern technology but the basic principle remains the same. A standardized clinical islet transplantation guideline was later established in 2000[11] that include proper islet handling, patient selection, and subsequent transplantation to the liver through the portal vein with $\geq$ 5000 islet equivalent/kg body weight.[12] The success rate in achieving glycemic control at one year post transplantation was reported at 44%[12] but declined overtime. Intra portal site is routinely used for unencapsulated islet transplantation, but the low oxygen tension within the liver vasculature, exposure to immunosuppressive drugs, liver ischemia from islet emboli, and immediate blood-mediated inflammatory response have led to research on transplantation with no immunosuppression.

Challenges in human islet isolation and transplantation

Exceptional advances have been made in the area of islet isolation but some barriers remain to its general use in the treatment of T1DM patients. Some of the challenges to this approach include limited number of human donors' pancreata for islet isolation. Also, ischemic injury from isolation and purification processes leads to islet loss, which necessitates the use of more than one donor pancreas to achieve a therapeutic transplant dose for each human recipient. Also, inadequacies in the currently available islet purification process exacerbate islet loss resulting in very low yields of islets during isolation.[13] In addition, immediate blood-mediated inflammatory response by immunologic cells to islet graft results in islet transplant failure. Human islets are very sensitive to low oxygen tension to which islets are exposed at the transplant site making transplant failure more likely.[14]

Alternative sources of islet for transplantation and their isolation

As a result of limited supply of human islets, alternative sources of islets have become imperative toward meeting demand for pancreatic islets for transplantation. Alternative sources for generating pancreatic islets/beta cells that are being investigated include human pluripotent stem cells—embryonic and adult somatic cells. Xenoislets, specifically porcine islets, constitute another alternative source of islets for human transplantation. Porcine islets are attractive because there is only one amino acid difference between human insulin and pig insulin with a single residue change at B30,[15] resulting in similar biological activities that justified the use of pig insulin to treat human diabetic patients for a very long time. Initial concern on possible transmission of retrovirus infection from porcine islet has been resolved.[16] Also, immune disguise of surface antigens on stem cell derived-beta cells and xenoislets to evade immune detection by allo and auto antibodies responsible for destruction of native and transplanted beta cells is also being explored.

A technology that has good potential to enhance the use of xenoislets in human diabetic patients is encapsulation prior to transplantation, which is capable of solving the problem of islet supply shortage. This approach may also help eliminate the need for chronic immunosuppression of transplant recipients, thus addressing the two major barriers to islet transplantation in patients.

Isolation of islets by encapsulation for immunoprotection

Islet graft triggers host immune response resulting in destruction of islet transplants. Thus, immune-protection of islets by means of encapsulation with semi permeable membrane allows the entry of oxygen and nutrients to the encapsulated cells and the exit of insulin and metabolic waste products while preventing the entrance of immune cells.

Traditional encapsulation approaches to islet cell immunoprotection

Macroencapsulation

Macroencapsulation of pancreatic islets is a method of encapsulation that involves the suspension of isolated (allogenic or xenogeneic) pancreatic islets in an implantable macrodevice.[17] A macrodevice measures more than 1 mm[18] and holds hundreds to thousands of islets, as illustrated in Figure 1(a). Macrodevices can be implanted in the extravascular or intravascular space, and come in various designs such as tubular hollow fibers, tubular ultra-filtrate chambers, planar device.[19,20] The merits of macroencapsulation include its ease of retrieval after implantation in case of graft failure or complications such as uncontrolled proliferation of islet—derived from human embryonic and pluripotent cells. Fabrication of macrodevices with thicker wall and thermoplastics offers good mechanical strength and ensures chemical stability for long-term implantation. Demerits of macroencapsulation include inadequate supply of oxygen and nutrients to the islets as a result of the relatively high islet density and diffusion distance from capsule to blood vessel. This puts islets at the risk for hypoxia, especially those cells at the center of the islet cluster, as they are the furthest from the nearest blood vessel. Hypoxia activates the apoptosis signal in beta cells[21]leading to decrease islet viability. In addition, the effective diffusional distance of the islet graft to the nearest blood vessel is 150–200 μm,[22] but the macrocapsule diameter is >1000 μm; this also causes a time lag in insulin response time to changes in host's blood glucose.[23] These issues have dampened the progress in the development of intravascular device until recently.

Microencapsulation

Microencapsulation is an encapsulation technique, which involves the enclosure of islet grafts in an often spherical microcapsule with a semi-permeable membrane, as illustrated in Figure 1(b). This permselective enclave allows the passage of oxygen, nutrients, and insulin while keeping out the host immunologic cells (leukocytes, macrophages). Numerous microcapsules are required to achieve normal blood glucose level. The size of a microcapsule ranges from 100 to ≤1000 μm in diameter with islet capacity of one or two per capsule and can be of various geometries.[24]

The therapeutic use of microencapsulation of islets was introduced by Lim and Sun in 1980[25] when they achieved normoglycemia in rats for three weeks by encapsulating islets in alginate/poly-L-lysine composite. The failure of the devices following this three-week period was attributed to poor biomaterial biocompatibility. Following this initial study, the use of microencapsulation in cell-based therapies for T1DM became a subject of interest and has become the most researched type of pancreatic islet encapsulation employed in the context of transplantation. Studies are replete of islet microencapsulation using natural (alginate, agarose, chitosan, gelatin, collagen) and synthetic (polyethylene glycol (PEG), polyvinyl alcohol(PVA) biomaterials[24] which have been tested in both animal and human models[26–28] with some short-term partial or complete control of blood glucose with or without immunosuppression. Large numbers of islet microcapsules are needed to achieve normal blood glucose and the site of implantation is usually in the peritoneal cavity, which is poorly vascularized relative to islets' high metabolic demands[29] which necessitate a plentiful oxygen supply.

Hydrogels are the most widely used means of microencapsulation, with alginate being the most commonly used biomaterial for enclosing cells. It can be sourced naturally from extracellular matrix of seaweed and is made up of guluronic and mannuronic acid with variation in the composition of these two monomers resulting in different properties for the microcapsules formed including the pore size, capsule strength, and biocompatibility.[30] Alginate is generally used because of its biocompatibility and gel-like properties when combined with divalent cations such as Ca,[2] Ba^{2+} Sr^{2+}, and Mg^{2+}.[31] There is a direct correlation between the purity of alginate and its degree of biocompatibility, with less purified alginate causing more fibrosis around capsules.[32] Alginate does not have adequate permselectivity and thus polyamides like PLL or PLO are used to improve the permselectivity of alginate[33] but they are strongly proinflammatory. External layering of the polyamide membrane with alginate to create alginate/polyamide/alginate microcapsule provides control over the pore size, thickness of the coating for better permselectivity, stability, and immunoisolation of the capsule.

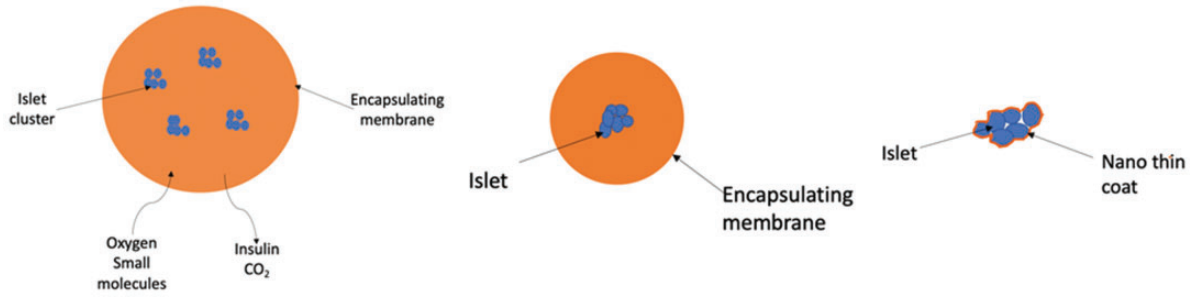

Figure 1. Illustration of the three different types of encapsulation for islets: (a) macroencapsulation showing multiple islet clusters within a macrocapsule equipped with a semi-permeable membrane; (b) microencapsulation of an islet cluster in a microcapsule within a semi-permeable construct; (c) nanoencapsulation in which each islet is encapsulated with a thin polymeric layer that protects the islet from immune attack. (A color version of this figure is available in the online journal.)

The merits of microencapsulation include increased surface to volume area, which ensures a more efficient transport of insulin and metabolites. Reduced cell density in the microcapsule improves viability of islet and minimizes islet loss from hypoxia. Disadvantages of microencapsulation include unreliable long-term stability of alginate hydrogel microcapsule membranes as a result of changes in pore size due to biochemical changes in the capsule microenvironment, which affects permselectivity.[34] Also, difficulty of retrieval from intraperitoneal cavity due to protein adsorption, fibrosis, and attachment to the omentum seems to occur most frequently with microcapsules $\leq 500\,\mu m$.[35] In addition, the uneven surface of smaller microcapsules that results from physical lodging of islets during encapsulation has been demonstrated to elicit an inflammatory reaction.[36] Studies have also shown that islet hypoxia occurs in microcapsules due to slow diffusion of oxygen and nutrients causing necrosis of islets and transplant failure, especially in larger microcapsules with diameter $\geq 500\,\mu m$.[37] Clumping of capsules on the pelvic floor from gravity[38] affects diffusion of nutrients and oxygen to the islets with resulting hypoxia in the generally poorly vascularized peritoneal cavity. Limited implantation sites for the many microcapsules are compounded by the large islet graft volume required to achieve therapeutic insulin level.

Nanoencapsulation

Nanoencapsulation of islets is an encapsulation technique that involves the application of biopolymer hydrogel directly on the surface of islet to create an ultrathin coating thickness[39] that molds to each islet, as illustrated in Figure 1 (c). This eliminates the diffusional distance between the encapsulating surface and the cell for better nutrient and insulin delivery. It ensures precise and uniform distribution of pore size for better selectivity. Nanoencapsulation can be achieved using a polyethylene glycol (PEG) conformal coating and layer-by-layer polymer assembly.[40] One merit of nanoencapsulation is the profound reduction in transplant volume achieved by virtually eliminating the void space between the islet and encapsulating membrane.[39] Elimination of the void space also improves glucose–insulin response time[41] and increases oxygen delivery to islets. Reduction in graft size also has the potential to allow for islet transplantation in narrow spaces like the portal vein which is commonly used as a transplantation site for unencapsulated islets.[12,42] Additionally, nanoencapsulation offers better control of uniform capsule thickness and pore size to enhance perm selectivity. It equally offers a way to incorporate tailored functionality to each film layer like immunocamouflage by masking surface antigens, angiogenicity, anticoagulability among other applications. Demerits include lack of long-term stability in single layer conformal coating, which unwinds as a result of islet membrane turnover.[41] In addition, the ultrathin coating does not withstand mechanical and biochemical stress, and incomplete coating and exposure of the islets triggers a host immune response.

Table 1 provides a summary of the merits and demerits of each of these three forms of encapsulation for islets.

New era of encapsulation

Encapsulation of islets cells is a work in progress and great strides have been made to improve islet viability and function in both *in vitro* and *in vivo* studies. Some of these developments are discussed below.

Reengineering of the encapsulation matrix to mimic the microenvironment of the native

Islet transplantation entails harvesting and isolation of islets from the native pancreas with processes to purify the isolated islets. This procedure strips the islets of extracellular matrix and supporting proteins, including other molecular components which make up the microenvironment.[43]The main proteins in the extracellular matrix are laminin, collagen, elastin, fibronectin as well as many other peptides entwined with glycosaminoglycans (like heparan sulfate, chondroitin sulfate, dermatan sulfate, or keratan sulfate),[43] which provide cell-to-cell signaling, stimulation of growth factors release, structural support, and stability important for islet viability and function.[44,45] Hence, designing an encapsulating hydrogel matrix that is similar to the microenvironment of the native pancreas is desirable. The matrix is reengineered to provide: (a) structural support for the islet, (b) biological cues for cell-to-cell interaction, and (c) immunoprotection/isolation for the islet.

Table 1. A summary of the merits and demerits of each of the three methods of encapsulation for islets.

Type	Merits	Demerits
Macroencapsulation	Easily retrievable. Mechanical strength and stability.	Inefficient transport of insulin, oxygen, nutrients, and metabolites to islets. Large transplant capsule diameter limits transplant sites. Inadequate biotolerance of biomaterials.
Microencapsulation	Improved transport of oxygen, insulin, and metabolites to islets than macrocapsule. Reduction in capsule diameter.	Not completely retrievable. Limited implantation sites Suboptimal diffusion of nutrients, oxygen. Inadequate biotolerance of biomaterials.
Nanoencapsulation	Significant reduction in transplant volume. Improved transport of oxygen, insulin, and metabolites than macro and microcapsule. More incorporation of functionalized capsule layers	Less mechanical stability. Inadequate biotolerance of biomaterials. Not easily retrievable.

The encapsulation matrix can be supplemented with specific ECM components or combinations of components to investigate the role of these proteins on islet function and viability. A study has demonstrated the role of laminin on microencapsulated porcine islets using ECM laminin peptide sequences RGD, LRE, YIGSR, PDGEA, and PDSGR in combination and singly to modify alginate microcapsule matrix. A capsule without covalently bound RGD was also used as control. Increased glucose-stimulated release of insulin and islet viability was observed in porcine islets encapsulated in RGD-bound alginate when compared to islets encapsulated in alginate non adhered to RGD and alginate adhered with other peptide sequences LRE, YIGSR, PDGEA, and PDSGR.[46] Enck *et al*.[45] modified alginate hydrogel microcapsule matrix by addition of decellularized ECM (dECM) components from human pancreas and observed improved stability of the morphology and mechanical strength of alginate microcapsules with significant increase in matrix stiffness of Sr^{2+} relative to Ca^{2+} crosslinked hydrogels (Figure 2), as well as improved function of encapsulated human islets (Figure 3).

Other studies have also incorporated specific ECM peptides or combinations,[46,47] with similar reports of improved islet function. However, while some combinations of ECM peptides may improve islet function and viability, other combinations decrease insulin production in beta cells. For example, *in vitro* studies of human fetal beta cells adhered to HTB-9 matrix, collagen IV, and vitronectin demonstrated reduced insulin gene transcription as compared with adult human beta cells.[47] In another *in vitro* study of microencapsulated human islets, collagen IV at concentration >50 μg/mL was found to have no effect on glucose-stimulated insulin secretion.[48]

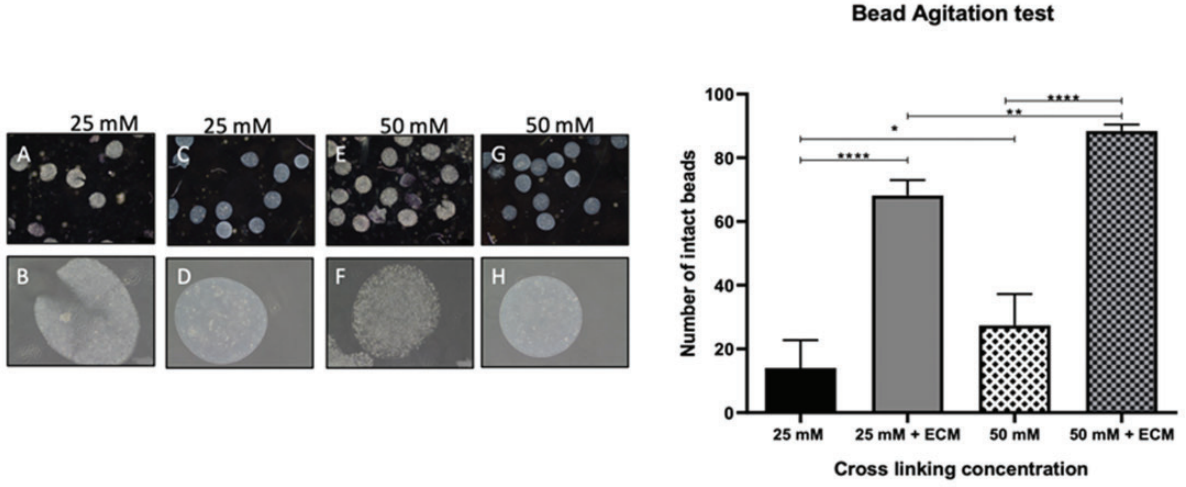

Figure 2 . Effects of alginate crosslinking cation and the incorporation of ECM into the encapsulation matrix on the morphological and rheological properties of a microcapsule construct. (a) Inverted microscopy images of alginate beads following 36 h of mechanical agitation. From left to right, the beads are crosslinked in 25 mM $SrCl_2$, 25 mM $SrCl_2$ with ECM-alginate, 50 mM $SrCl_2$, and 50 mM $SrCl_2$ with ECM-alginate. (b) Number of beads intact after mechanical agitation. Initial count of 100 beads were made with or without ECM and crosslinked with either 25 mM or 50 mM $SrCl_2$. Student's *t*-test (mean ± SD, $n = 5$, $^*P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$). Reprinted with kind permission from Enck *et al*.[45]

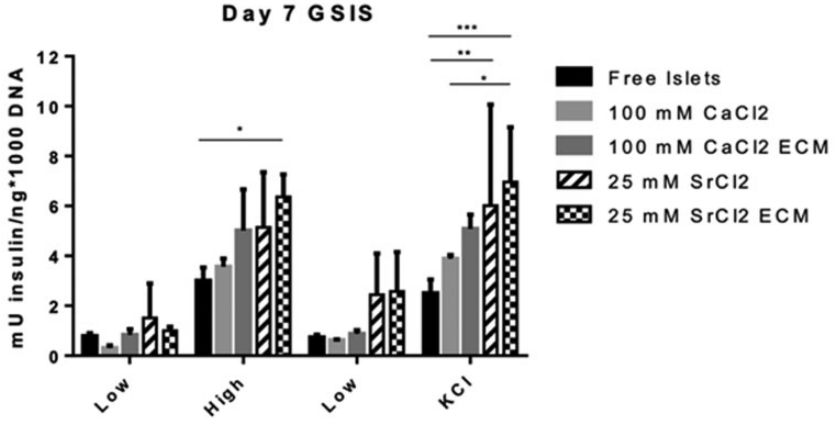

Figure 3. Effects of alginate crosslinking cation and the incorporation of ECM into the encapsulation matrix on encapsulated islets glucose-stimulated insulin secretion (GSIS) results on day 7 post-encapsulation one-way ANOVA (mean ± SD, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $n = 3$). Reprinted with kind permission from Enck *et al*.[45]

Incorporation of oxygenation materials into the construct

Islets have high metabolic demands and require adequate oxygen to function properly. Hypoxia during isolation, encapsulation, and post transplantation ischemia can lead to as much as 60% islet loss before revascularization of islet graft by angiogenesis, which can take up to two weeks.[44] Macrodevices with refillable gas chambers can be cumbersome and may result in patient noncompliance. Oxygen-generating compounds such as calcium peroxide (CPO) and sodium percarbonate (SPO) commonly referred to as particulate oxygen-generating substances (POGS), which become reactive in the presence of water to produce oxygen provide an alternative approach for oxygen supply to reduce the effect of hypoxia on islets with positive results reported. However, free radicals are also formed during oxygen generation from POGS, which can have deleterious effects on the islets when high concentrations of these oxygen generating compounds are used. In a study by a research group, an increase in glucose-stimulation index (GSI) of porcine islets when a defined amount of SPO was added during islet isolation, purification, and culture was observed. They also reported an increase in porcine islet viability and function of coencapsulated islets and CPO after seven days.[49]

In another *in vitro* study, the Oxysite (calcium peroxide in polydimethylsiloxane) macroencapsulation device was used to show enhanced function and viability of mouse beta cells, rat, and human islets compared to control islets without Oxysite. The polydimethylsiloxane served to neutralize free radicals. *In vivo* studies using mouse beta cells within Oxysite macro device in immunocompetent streptozotocin-induced diabetic mice were reported to demonstrate improved graft function with shorter time to achieve and maintain euglycemia for over 30 days.[50] *Ex vivo* examination following explantation of the mouse beta cells showed increased insulin secretion and glucose-stimulated insulin response.[50] Silicone-calcium peroxide with extracellular hemoglobin coencapsulated with pig islets was described to have improved islet viability and insulin secretion. Hemoglobin served as an oxygen carrier and also reduced oxidative stress that can be caused by oxygen-free radicals.[51] Research is ongoing in the context of clinical utilization of oxygen-generating materials in encapsulation.

Post-transplant vascularization strategies

Following transplantation of islets, there is an interim period of up to two weeks before the reestablishment of blood supply to the graft by neoangiogenesis.[52] Engrafted islets in the meantime depend on diffusion of oxygen and nutrients from nearby blood vessels for metabolic activities with effective diffusional distance being $\leq 200\,\mu m$. This often creates low oxygen tension in the islet microenvironment with risk for graft ischemia and death.[29,53]Loss of islet viability due to hypoxia has been a persistent challenge for long-term survival of encapsulated islet post transplantation, and as a result, strategies to stimulate new blood vessel growth and consequently improve islet survival are being examined.

Co-encapsulation of proangiogenic factors with islets in hydrogel has been used to enhance revascularization of islet grafts. Opara *et al*. studied the co-encapsulation of proangiogenic protein, fibroblast growth factor-1 (FGF-1) with heparin in the outer alginate coating of alginate poly-L-ornithine alginate (APA)) microcapsules. They observed sustained release of FGF-1 with increase in angiogenesis and maintenance of its biological activity for 30 days,[54]while *in vivo* studies with islet isografts and allografts in diabetic rats showed increased blood vessel formation and islet viability.[55]

The viral vector transduction method is another approach to enhance islet graft neoangiogenesis by increasing gene expression of proangiogenic factor, vascular endothelial growth factor (VEGF). *In vitro* investigation of transcribed viral mRNA in islet grafts to stimulate angiogenesis has been demonstrated to result in a transient increase in vascular endothelial factor leading to a significant increase in vascularization of transfected syngeneic mice islet transplant and also in xenotransplant of transfected human islet grafts in severe combined immunodeficient mice.[56] Another *in vivo* study with non-viral plasmid vector encoding VEGF-A in human islet graft in mice liver reported euglycemia, which was attributed to rapid revascularization of islet engraft.[57]

Alternative transplant sites to the traditional intraperitoneal space for encapsulated islet transplantation

For encapsulated islets, the routinely used liver transplantation site through the portal vein for unencapsulated islets is not applicable because of the large volume of the transplant. The peritoneal space and subcutaneous space are commonly used for encapsulated islet transplantation, but are not well vascularized, resulting in reduced oxygen and nutrient flow, which is detrimental to islet. The omentum has been investigated and has shown promise as a viable alternative transplant site. A study using microcapsules implants of allogeneic islets in rat omental pouches reported significant islet survival post implantation.[54] Intravascular macrocapsule transplant of xenoislets from rabbits has also demonstrated islet survival and function in T1DM patients.[58]

It remains to be determined if any of the alternative sites mentioned in this review would be useful in encapsulated islet clinical transplantation. Encapsulated islet viability in larger animal models (non-human primates, pigs, dogs) is more challenging compared to rodents due to robust immune response causing more fibrosis of encapsulating device impairing nutrient exchange.[59] A recent study using triazole-modified alginate hydrogel appear to prevent excessive fibrosis common with larger animal models (non-human primates) and may be useful in prolonging encapsulated islet graft.[60]

Clinical translation of encapsulated islets technology – The bioartificial pancreas

A bioartificial pancreas is defined as a pancreatic islet construct based on encapsulation of islet cells within a semipermeable membrane so that the cells can be protected from the host's immune system while they secrete insulin to regulate blood sugar. This is to be distinguished from an artificial pancreas, which is an insulin delivery system accomplished by an insulin pump integrated with a glucose sensor with no living cell involvement. A bioartificial pancreas can be either a macrocapsule construct, microcapsule, or nanocapsule constructs.

Macroencapsulated islets

Preclinical studies abound demonstrating islet function in macrocapsules, but clinical trials are currently sparse. A study demonstrated the viability of macroencapsulated islets after two weeks in a hollow fiber membrane when implanted subcutaneously in nine patients, three for each group of diabetic T1DM, T2DM, and non-diabetic recipients.[61] Beta O_2 device which supplies oxygen to islet graft was utilized to investigate intraperitoneal islet allotransplantation in a non-immunosuppressed long-term type 1 DM patient with report of improvement in HbA1C and slight reduction in insulin requirement for 10 months. A functional islet graft without fibrosis was retrieved following explantation at the end of the clinical trial.[62] Intravasular xenotransplant of islets from rabbit fetuses in 73.2% of T1DM recipients showed reduced insulin requirements and stable glycemic control for two years without immunosuppression.[58] These examples provide evidence of the potential application of the macrocapsule construct in future cell-based therapy and probably in xenotransplantation.

Microencapsulated islets

Advances have been made using microencapsulated islets in clinical application since the introduction and application of the concept. Soon-Shiong *et al.*[27] reported transient euglycemia in an immunosuppressed diabetic patient. Calafiore carried out a successful clinical allotransplantation of microencapsulated islet in non-immunosuppressed T1DM patients with reduction in exogenous insulin requirements and hypoglycemic episodes.[28] Clinical allotransplantation of microencapsulated islets has also been demonstrated to be safe[63] with long-term stable blood glucose, reduced insulin requirements, and non-sensitization of host to islet graft antigens[64] in non-immunosuppressed recipients.

The first clinical trial of xenotransplantation of microencapsulated islet carried out in New Zealand demonstrated the safety of using porcine islets without transmission of porcine endogenous retroviruses to 14 non immunosuppressed type 1 diabetic recipients which has been a concern in porcine islet xenotransplant,[16] although islet graft function was low with minimal reduction in HbA1c and insulin dose requirement. Another clinical trial carried out with porcine islet transplantation showed significant improvement in diabetic recipients HbA1c $>$ 600 days following a two-time transplant of 10,000 IEQ/kg porcine islets at three months intervals without immunosuppression.[65] Long-term safety of porcine xenograft in recipients has also been confirmed.[16,66]

Nanoencapsulated islets

Clinical utilization of nanoencapsulated islets is attractive because of the significant reduction in islet transplant volume, improved diffusional limitations, and reduced inflammatory immune response by modulation of islet microenvironment through functionalized nanolayers. However, the only clinical trial in the US utilizing nanoencapsulation involves two T1DM patients implanted with allogeneic islets into the subcutaneous tissue in the back and abdomen with resulting reduction in hypoglycemic and hyperglycemic episodes, but exogenous insulin independence was not achieved in either patient throughout the duration of the trial at six and four months, respectively.[58]

Conclusions

Encapsulation of islets is a pivotal innovation with obvious potential capability to enhance transplant survival and secretory function. Encapsulated islets equipped with adequate barrier to host immune cells and antibodies would advance islet transplantation without use of toxic immunosuppressive drugs to prevent transplant rejection while addressing donor islet shortage. Nevertheless, more advances are needed to achieve a better islet immunoisolation without impeding nutritional transport and therapeutic delivery of insulin within appropriately designed encapsulation matrix that resembles the native pancreatic microenvironment. Also, more studies of efficacy in preclinical trials with larger animal models are needed as *in vitro* and preclinical rodent studies often do not always translate to human response. In conclusion, careful optimization of the encapsulation technology will accelerate its clinical translation and conventional use as a therapeutic option in diabetes mellitus.

AUTHORS' CONTRIBUTIONS

AO and AJ generated the first draft of the manuscript and formatted it according to journal specifications for submission. SDJ reviewed and edited the manuscript. EO generated the review outline, reviewed, and edited the manuscript.

DECLARATION OF CONFLICTING INTERESTS

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ORCID iDs

Alec Jost https://orcid.org/0000-0003-2628-3072
Sam Dagogo-Jack https://orcid.org/0000-0001-5318-9677

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