Title
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Journal
The Review of Diabetic Studies, 11(1)

ISSN
1613-6071

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Publication Date
2014

DOI
10.1900/rds.2014.11.84

Peer reviewed

Islet and Stem Cell Encapsulation for Clinical Transplantation

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Acknowledgement

This review was funded by the University of California Irvine, Department of Surgery. Images of the multi-layer alginate capsules and nanoencapsulated islets were generously provided by Dr. Paul de Vos and Dr. Camillo Ricordi in personal communication with Dr. Jonathan RT Lakey.

Disclosures: The authors of this review declare no conflict of interest in its preparation.

Abstract

Over the last decade, improvements in islet isolation techniques have made islet transplantation an option for a certain subset of patients with long-standing diabetes. Although islet transplants have shown improved graft function, adequate function beyond the second year has not yet been demonstrated, and patients still require immunosuppression to prevent rejection. Since allogeneic islet transplants have experienced some success, the next step is to improve graft function while eliminating the need for systemic immunosuppressive therapy. Biomaterial encapsulation offers a strategy to avoid the need for toxic immunosuppression while increasing the chances of graft function and survival. Encapsulation entails coating cells or tissue in a semipermeable biocompatible material that allows for the passage of nutrients, oxygen, and hormones while blocking immune cells and regulatory substances from recognizing and destroying the cell, thus avoiding the need for systemic immune therapy. Despite advances in encapsulation technology, the developments have not yet been meaningfully translated into clinical islet transplantation, for which several factors, including graft hypoxia, host inflammatory response, fibrosis, improper choice of biomaterial type, lack of standard guidelines, and post-transplantation device failure, are to blame. Several new approaches, including the use of porcine islets, stem cells, development of prevascularized implants, islet nanocoating, and multilayer encapsulation, continue to generate intense scientific interest in this rapidly expanding field. This review provides a comprehensive update on cell encapsulation of islets as a treatment modality in type 1 diabetes, including a historical outlook as well as current and future research avenues.

Keywords: type 1 diabetes, stem cells, islet transplants, encapsulation, tissue engineering

Abbreviations

ADSC – adipose-derived stem cells

a-FGF - acidic fibroblast growth factor

AN69 - acrylonitrile 69

APA - alginate poly-L-lysine alginate

CITR - Collaborative Islet Transplant Registry

EuroSPK - European Study Group in Simultaneous Pancreas and Kidney Transplantation

FGF-1- fibroblast growth factor 1

HbA1c - hemoglobin A1c

HEMA - 2-hydroxyethyl methacrylate

hESC - human embryonic stem cells

ICA - islet-like cell aggregates

IEQ - Islet equivalent

IPITA - International Pancreas and Islet Transplantation Association

iPSC - induced pluripotent stem cells

IPTR - International Pancreas Transplantation Registry

ITA - islet transplantation alone

LBL - layer-by-layer

M:G - mannuronate: guluronate

MIN6 - mouse insulinoma 6

NOD - non-obese diabetic

PAK - pancreas after kidney transplantation

PDMS - polydimethylsiloxane

PEG - polyethylene glycol

PEG–PLGA - polyethylene glycol-poly lactic-co-glycolic acid

PGA - polyglycolic acid

PLL - poly L-lysine

PPB - poly (l-lysine) - g-poly (ethylene glycol) (biotin)

PTA - pancreas transplantation alone

PTFE - polytetrafluoroethylene

PU-PVP-IPN - PolyUrethane-Poly Vinyl Pyrrolidone-InterPenetrating Network

PVA - polyvinyl alcohol

SA - streptavidin

SOP - standard operating procedure

SPK - simultaneous pancreas and kidney transplantation

STZ - streptozotocin

T1D - type 1 diabetes

1. Introduction

Type 1 diabetes (T1D) is an autoimmune disorder characterized by the rapid destruction of β-cells within the pancreas resulting in an inexorable decline of insulin secretion culminating in complete insulin deficiency [1]. The primary treatment modality for T1D today is strict glycemic control through injectable exogenous insulin, administered subcutaneously. A surgical alternative to exogenous insulin is clinical islet transplantation, a procedure that attempts to replenish the depleted β-cell reserve by transplantation of isolated donor islets into the patient's portal vein. Over the last decade, this procedure has undergone several modifications and refinements, such that current recipients are able to stay independent of insulin for prolonged periods of time. Unfortunately, the lack of organ donors of sufficient quality, the need for multiple donors per patient, inconsistent islet yields, and the need for immunosuppressive therapy continue to hamper further progress [2]. Encapsulation of islets prior to transplantation could potentially address some of these problems.

Cell encapsulation is a novel concept in which cells are encased within a biocompatible matrix. The primary role of encapsulation is to create a barrier against large immune cells and cytotoxic molecules, which could potentially injure the cell, thus avoiding rejection while still allowing the active diffusion of oxygen, micro- and macronutrients, and hormones (**Figure 1**). Although the idea was described as early as the 1930's, no notable achievements occurred until the last decade. This review aims to provide an update on this new and rapidly evolving area of islet research while including relevant historical events, current trends in islet research, and future

directions in this exciting field with the hope of ultimately utilizing islet and stem cell encapsulation to cure T1D.

2. History of islet transplantation

In the annals of islet transplantation, the year 1894 will be remembered as the year in which islet transplantation was first attempted as a treatment for T1D. That year, Dr. Williams, a British physician, attempted to cure a young diabetic by injecting a concoction of pancreatic cells obtained from sheep, under the patient's skin. Unfortunately, the treatment did not work, and the patient slipped into a diabetic coma and died two days later [3]. Following this failed attempt, no inroads were made into devising a surgical cure for T1D until the 1960s, when Dr. Kelly at the University of Minnesota attempted to transplant a segmental pancreas graft and a kidney simultaneously, from a cadaver donor into a diabetic patient with end-stage renal disease. Although the 28-year old recipient remained insulin-free for only 6 days post transplantation, rejected both grafts within 60 days of the surgery, and died of a pulmonary embolism less than two weeks after the organs were explanted, the surgical team had proven that this complicated procedure was technically feasible [4].

Unlike Dr. Kelly's pioneering surgery, where a segmental pancreatic graft was preferred, Dr. Lillehei transplanted the whole pancreas and attached duodenum either with (9 cases) or without the kidney (4 cases). This modified technique demonstrated prolonged pancreas graft function, in one case for nearly one year [5]. These trials were quickly followed by similar trials in South America [6-8], the US [7, 9], and Europe [7]. Since none of the trials demonstrated graft function for more than a year, and since the graft duodenum was noted to be robustly antigenic, techniques involving the transplant of a segmental pancreatic graft were developed in the 1970s.

The introduction of a novel immunomodulator, cyclosporin A, in clinical transplantation [10], the formation of the International Pancreas Transplantation Registry (IPTR), and the organization of scientific meetings in Spitzingsee, Germany, in the early 80's, which led to the creation of IPITA (The International Pancreas and Islet Transplantation Association) and EuroSPK (The European study group in simultaneous Pancreas and Kidney Transplantation) [11, 12], were three major events that contributed immensely to the development of pancreas transplantation. In the 1970's, surgeons at the University of Minnesota were performing pancreas after kidney (PAK) transplantations in diabetic patients, but by the 1980's, they had commenced segmental pancreas grafting for pancreas transplantation alone (PTA) [13].

In 1983, Dr. Sollinger reported a urinary drainage technique as a method for managing pancreatic exocrine secretions. The low incidence of surgical complications [14, 15] using this method, and the added advantage of being able to monitor graft rejection using urinary amylase monitoring, led to the rapid adoption of bladder drainage using whole pancreas as the standard technique for the surgical management of T1D. The whole pancreaticoduodenal transplant with enteric drainage, originally described by Lillehei [5], was perfected during the mid-80s by Starzl and associates [16], and remained the most popular technique for a decade. This technique subsequently became well established for use in simultaneous pancreas and kidney transplantation (SPK) with concomitant immunosuppressive therapy. The same technique has been modified for use in several transplantation procedures, especially in PAK transplantation and PTA. The duodeno-duodenal enteric anastomosis technique is, however, a high-risk

procedure which requires the use of anti-thrombotic drugs to prevent ischemic reperfusion injuries [17].

Before 1999, results from islet transplantation alone (ITA) were poor and not comparable to survival rates for solid organ (whole or segmental pancreas) grafts. According to the 2005 Islet Transplantation Registry (ITR) report released in May 2005 at the 10th IPITA Congress, only 10% of islet transplant recipients demonstrated insulin independence at the end of one year, and even this varied depending on the center where the procedure was performed (IPITA 2005, Geneva). Since the first clinical trial where human islet allotransplantation was attempted in 1974, roughly 364 such procedures had been reported in 1999 from around 15 centers all over the world.

In March 1999, a new protocol for clinical islet transplantation using a glucocorticoid-free immunosuppressive regimen, developed in Edmonton, Alberta in 2001, demonstrated sustained insulin independence in seven human subjects for a period of almost one year [18]. When centers reported that 80% of patients treated using the steroid-free Edmonton regimen remained insulin-free after one year post transplantation, there was significant interest generated as this represented a definite improvement over previous immunosuppressive regimens, most of which included glucocorticoids which had demonstrated significant islet toxicity. Ten other centers attempted to replicate this initial success, but reported disappointingly low long-term exogenous insulin independence (> 4 years), with a return to hyperglycemia and insulin dependence despite immunosuppression [19]. Of the nine largest centers where human islet allotransplantation was carried out using the Edmonton protocol, only three were able to demonstrate a 65% insulin

independence rate at one year after the procedure, while some centers reported success rates as low as 23% [20].

This has encouraged researchers to scout for other, non-hepatic transplant sites where presumably the early islet destruction—secondary to an intense inflammatory response, inadequate graft vascularization, and toxic levels of immunosuppressant drugs—characteristic of intra-hepatic islet allotransplantation can be avoided [21]. A comprehensive review by Barton *et al*. using data obtained from the Collaborative Islet Transplant Registry (CITR), involving a total of 677 recipients who underwent allogeneic islet transplantation between 1999 and 2010 [22], has shown that exogenous insulin independence three years after transplantation improved from 27% between 1999-2002 to 44% between 2007-2010. Between 2007 and 2010, islet graft survival at 1 year (92%) and 3 years (83%) is comparable, if not slightly superior, to survival rates demonstrated with whole pancreas transplants (80% and 61% at 1 year and 3 years, respectively). Better graft survival rates noted in recent years are attributed to advanced and targeted immunosuppressive strategies which conferred greater protection to the transplanted islets.

To avoid graft rejection, islet transplant centers have also implemented various immunosuppressive regimens. This immunosuppressive therapy is a lifelong requirement and has been demonstrated to result in numerous deleterious effects to the patient, while also adversely impacting graft function and viability [23, 24]. Encapsulation of transplanted cellular grafts within biocompatible materials has been proposed as a viable treatment option for allogeneic human islet transplantation with the goal of eliminating the need for chronic

immunosuppression, thus obviating the attendant adverse effects. Encapsulation simply involves enclosing tissues within immunoprotective hydrogels to achieve immunoisolation to prevent graft rejection. Various encapsulation technologies have been previously used in the fields of anemia, dwarfism, neurodegenerative diseases, liver failure, chronic pain syndromes, epilepsy, and diseases of the parathyroid glands [25-29]. Despite intra-portal infusion of islets having been established as the standard of care in the treatment of T1D, maintaining long-term graft viability while avoiding toxic systemic immune therapy has only been accomplished through encapsulation [30, 31].

3. Advances in encapsulated islet technology

3.1 Animal and human trials

In 1933 Bisceglie demonstrated that a membranous polymer structure, containing mouse tumor cells, can be transplanted safely into the abdominal cavity of a guinea pig while successfully evading the immune system and simultaneously preserving the viability of the encapsulated cells for prolonged periods of time. Today, this is widely regarded as the first scientifically documented attempt to encapsulate cells in bio-artificial membranes [32].

Alginate is a commonly used term to refer to a family of complex polysaccharides commercially extracted from seaweed, including fast-growing kelp (brown algae) and certain bacteria (Pseudomonas and Azotobacter). It is composed of linear binary copolymers of β-D-mannuronic (M) and α-L-gulucorunoic (G) acid and exhibits a favorable immunologic profile after it undergoes extensive purification [33-35]. Over 30 years ago, in 1980, Lim F *et al.* demonstrated that intra-peritoneal transplantation of 2,000-3,000 islet equivalents (IEQ) encapsulated in

alginate-poly-L-lysine alginate (APA) microcapsules into diabetic rats was able to reverse STZ-induced hyperglycemia for a period of up to 21 days post transplantation. When unencapsulated islets were transplanted, there was a return to hyperglycemia after a period of only 8 days [36]. Over the last two decades, numerous studies have demonstrated that encapsulated syngeneic and xenogeneic islets continue to function for prolonged periods of time after successful transplantation into small [37-42] and large animals [43-46]. Encouraging results, including prolonged graft survival and a reduction in HbA1c levels and daily insulin requirements have even been demonstrated in a few early-phase human clinical trials [47-50]. Similar results have been obtained using various biomaterial implant devices including microcapsules made of 5% agarose [51], polyethylene glycol (PEGylated islets) [52], and polyethylene glycol-poly lactic-co-glycolic acid (PEG–PLGA) nanoparticles [53]. Alginate has become heavily favored for use in microencapsulation because of its superior biocompatibility, hydrophilicity, simple gelation process, stable architecture, abundant availability, relative ease of procurement, low manufacturing costs, and prolonged stability *in vivo*. Alginate microencapsulation is a technique in which each islet is individually enclosed in an alginate microcapsule (**Figure 2E and F**). Alginate has also been used in macroencapsulation, where the biocompatible device can contain multiple islets (**Figure 2A-D**).

While alginate encapsulated islets have been successfully transplanted into the peritoneal cavity of canine subjects [54], under the skin and kidney capsule in non-human primate [43] and cynomolgus monkey recipients [45] with moderate success, these studies have been lacking in consistency. Regardless of this drawback, these large animal studies have paved the way for several researchers to proceed to phase one and two clinical trials in human subjects.

In 1994, the world's first clinical trial using encapsulated human islets was conducted when a 38-year old diabetic male patient suffering from end-stage kidney disease was transplanted with encapsulated human islet allografts while simultaneously being administered low-dose immunosuppression [55]. He received 10,000 islet equivalents per kilogram (IEQ/kg) body weight encapsulated within alginate microcapsules. Six months later, he received a second infusion of 5,000 islet equivalents per kilogram (IEQ/kg) body weight. He recovered from the surgery without any complications and was insulin-free 9 months after he received the first transplant [55]. Other studies were less successful. In 2006, Calafiore *et al*. reported significantly lower exogenous insulin requirements and improved glycemic profiles in two subjects that received encapsulated human islets. While this was encouraging, it must be noted that the subjects did not achieve insulin independence [48].

Living Cell Technologies, a company based in New Zealand, harvests pancreases from fetal pigs housed at a pathogen-free farm on Auckland Island, following which the islets are isolated in a pathogen-free facility, encapsulated within alginate microcapsules, and injected intraperitoneally into T1D human recipients. They have demonstrated significant success in the field of encapsulated islet transplantation. Several early-phase human trials have demonstrated a significant reduction, 40% of pre-transplant levels, in the number of hypoglycemic episodes. Patients also reported reduced insulin requirements and 2 of the subjects enrolled in the study reverted to insulin independence 4 months after transplantation [45, 56, 57].

Despite these promising achievements, most recipients of encapsulated islets fail to achieve sustained insulin independence and many studies demonstrate disappointing results, ranging from relapse into hyperglycemia and return to pre-transplant insulin requirements [57] to a complete failure to achieve insulin independence or even a reduction in insulin requirements, despite the presence of detectable C-peptide levels [50]. Pericapsular fibrosis seems to be a significant problem that results in increased failure rates after transplantation of microencapsulated islets [50, 58-60].

In vivo studies have consistently demonstrated that some biomaterial implants used in encapsulation impact implant survival more positively than others. King *et al.* demonstrated that islets encapsulated in poly L-lysine- (PLL-) free high mannuronate alginate (high M) demonstrated prolonged periods of sustained normoglycemia (up to 8 weeks) as compared to capsules composed of high guluronate (high G) alginate [61]. However, Espevik *et al*. demonstrated that capsules made of high M alginate stimulated monocytes to produce TNF-α, IL-1, and IL-6, all of which are pro-inflammatory cytokines that would adversely impact islet survival [62]. On the other hand, high G alginates did not demonstrate similar pro-inflammatory characteristics [63]. Lanza *et al*. conducted studies comparing microcapsules of different alginate concentrations and demonstrated that improved capsule stability and prolonged graft function and survival could be achieved by simply altering the concentration of alginate (from 0.75% to 1.5%) used for encapsulation with porcine and bovine xenografts transplanted into immunocompetent rats [64].

The length and sequence of mannuronate and guluronate chains and ratio of mannuronate to guluronate (M:G ratio) in alginate hydrogels has been demonstrated to determine the mechanical strength, elasticity, durability, permeability, and swelling characteristics of the alginate [33, 65]. In addition, several chemical changes to the alginate composition have either positive or undesirable effects on its biocompatibility. The use of multivalent cations (Ca^{2+}, Fe^{3+}, $Ba^{2+)}$, polycations (e.g., poly-L-lysine or poly-L-ornithine) and poly-electrolytes in alginate synthesis as a technique of modifying its chemical properties has been extensively studied [66, 67]. Alginate poly-L-lysine alginate (APA) capsules [68, 69] provide a high degree of permselectivity (selective blocking of antibodies from being able to enter microcapsules), better stability, and mechanical strength, but also result in increased pericapsular cellular overgrowth [70] greater antigenicity [71, 72], and macrophage activation [73, 74] resulting in a dense fibrotic overgrowth surrounding the capsules [75].

These disadvantages can be avoided by cross-linking the alginate using a multivalent cation like barium, which results in the formulation of highly elastic alginate capsules with greater stability and better mechanical strength [67, 76]. Barium cross-linking has been demonstrated to be significantly less immunogenic [77] than alginate-PLL (or other polycation-linked alginate capsules) and also provides sufficient protection from antibody and cytokine mediated islet-injury, despite being more permeable to IgG antibodies than alginate-PLL capsules [78]. These advantages have led to the widespread use of barium cross-linked alginate in islet encapsulation. Although alginate remains the most popular hydrogel of choice, agarose [79, 80], chitosan [81], methacrylic acid [82], methyl methacrylate [83], polyamide [82, 84], polyvinyl alcohol (PVA) [85], polyethylene glycol [86, 87], 2-hydroxyethyl methacrylate (HEMA) [88, 89], and AN69 (a

copolymer of acrylonitrile and sodium-methallyl sulfonate) [90], have been used in islet encapsulation studies with limited success.

3.2 Biomaterials in transplantation

Devices used in cell encapsulation today can broadly be classified into macroscale, microscale, and nanoscale devices, the variety thereof is shown in **Figure 2**. These implantable devices could be implanted in intravascular or extravascular sites anywhere in the body.

Intravascular devices. Intravascular devices contain islets encapsulated within hollow biocompatible tubes or fibers attached to the recipient's vascular system [91, 92]. They present several advantages over extravascular macro capsules, namely, better access to nutrients and oxygen, immediate recognition of changes in glucose, and higher diffusion facilitated by blood flow. However, their tendency to develop thrombi at anastomosis sites and the need for systemic anticoagulation with its attendant adverse effects [91, 92] make them poor candidates for widespread use in clinical islet transplantation.

Extravascular devices. Research into extravascular macroencapsulation devices [45, 93-100] has been progressing at a steady pace. The hemorrhagic complications seen with implantable intravascular devices are not noted with extravascular implants and consequently these devices have been studied more extensively. They may be broadly classifiable into tubular and planar devices based on their morphology.

Tubular devices. Numerous studies using islet-containing extravascular tubular chambers and sealed hollow fiber devices [99, 101-105], performed during the last two decades, have demonstrated shorter graft survival times when compared to similarly constructed intravascular devices. This was attributed to inadequate oxygen and nutrient diffusion within these devices when compared to intravascular devices. XM-50 Amicon hollow fiber macrocapsule implants (Amicon Corp, Danvers, MA), containing xenogeneic human [106] or canine islets transplanted into STZ-diabetic pigs and rodents respectively, demonstrated that the peritoneal cavity was the best transplant site with minimal fibrosis even 5 months post transplantation, despite no immunosuppression [107]. When transplanted into diabetic dogs, these devices demonstrated a 50% success rate in achieving insulin independence for a period of 51-82 days, demonstrating their efficacy in large animal models [108].

Islet-containing hollow fiber implants with smooth outer surfaces demonstrated better immunoisolation and glycemic control when implanted subcutaneously, with minimal fibrotic response and implant failure as compared to implants with rough or fenestrated outer surfaces [107-110]. Prevost *et al*. [111] reported that STZ- diabetic rats transplanted with AN69 hollow fiber implants containing syngeneic islets demonstrated euglycemia for 10 weeks post-transplantation. No host reaction to the implant was evident except for a thin layer of fibroblasts. Studies have also demonstrated that these fibers have neovascularization potential [112], similar to intraperitoneally transplanted, smooth surface regenerated cellulose fibers [113]. A recent *in vitro* study even suggested that islets encapsulated in hollow fibers demonstrate adequate oxygenation, comparable to levels found within microcapsules [114]. Hollow fiber devices are injectable, easily retrievable, durable, and easily adaptable for subcutaneous implantation.

However, they are also highly susceptible to damage after transplantation *in vivo* and require a large dose of islets to achieve complete insulin independence [98], which limits their widespread applicability.

Planar devices. Planar devices consist of islets encapsulated within two circular or rectangular flat sheets fastened to make a sealed chamber. It is believed that this configuration confers better stability than hollow fiber chambers and attenuates graft hypoxia by improving oxygen supply to the entire graft. These devices are implanted either in the subcutaneous tissue or in the peritoneal cavity because of their configuration and macroscopic size. In the case of prevascularized devices, the former site is preferred, as a second procedure is often needed to seed the device with islets weeks or months after the initial surgery. However, planar implants seldom remain in their original configuration after implantation [115] and studies using these devices have demonstrated graft failure secondary to the formation of a dense pericapsular fibrotic overgrowth [116, 117]. Poor oxygen and nutrient diffusion across the membranes leading to compromised islet viability, suboptimal graft function, and graft failure limit their ability to sustain insulin independence for prolonged periods of time.

Despite these disadvantages, their easy retrievability after implantation for further evaluation and their utility in performing *in vivo* islet viability [118] and implant biocompatibility studies and in the study of the diffusional, and immunogenic properties of a particular biomaterial implant [119, 120] has led to their widespread use in numerous *in vivo* islet encapsulation studies. Certain bilayered planar devices such as the Boggs chamber and the Theracyte device (**Figure**

2C) can be modified to promote vascularization while simultaneously providing effective immunoisolation [92, 121].

Prevascularized devices. A 'prevascularized' device is designed to increase vascularity at the transplant site by the local administration of vascular growth or trophic factors, or by the induction of neovascularization by device pre-implantation followed by islet seeding several weeks later (**Figure 2A and B**). Prevascularization has been evaluated as a possible solution to overcome the diffusional limitations noted with planar devices and to mimic the native microarchitecture of the islets, where β-cells enjoy intimate contact with the surrounding microvasculature [122, 123]. Despite studies reporting successful implantation of prevascularized islet-containing devices in the omentum and other sites in the peritoneal cavity, subcutaneous implantation remains the most attractive location and represents the safer, less invasive alternative with minimal adverse effects that also allows for continuous monitoring and effortless device retrieval [124, 125].

Pore size. Choosing the appropriate pore size is vital for the success of any bio-artificial encapsulation device. An exceedingly small pore size may impede inward nutrient and oxygen diffusion and outbound insulin and metabolite diffusion from the islet-containing inner space of the planar device, but a particularly large pore size may allow unwanted immunoglobulins and other cytotoxic chemicals to enter this space, leading to islet injury and destruction. Thus, the most important criterion in designing a functional islet encapsulation device is appropriate pore size. Colton *et al*. studied the influence of pore-size on membrane permeability by seeding mouse insulinoma cell clusters (MIN6) encapsulated in 1% agarose, 0.005% HEMA, and 0.15

mg/ml collagen [119] into planar Nuclepore [126] membrane devices. Devices with pore sizes ranging from 0.1 mm up to 0.6 mm were transplanted intraperitoneally into diabetic Wistar rats and compared. While devices with 0.1 or 0.2 mm pore-sized membranes demonstrated sustained reversal of hyperglycemia for at least 3 months [127], those with larger pore sizes demonstrated a partial reversal that was not sustained for more than 3 weeks after implantation.

Studies have also demonstrated that membrane diffusion and permeability in planar devices can change after exposure to host defenses. Kessler *et al*. performed experiments where a protein coat was applied to the encapsulation device before *in vitro* tissue culture in an attempt to mimic an *in vivo* host environment [120]. Membrane permeability to glucose and insulin was assessed during culture and compared with results obtained with devices implanted intraperitoneally in rats and retrieved one week after implantation. No cellular inflammation or necrosis, cell adhesion or fibrin deposits were noted in the non-coated device implanted in-vivo. However, protein adsorption onto the *in vivo* implanted membranes was greater than the amount adsorbed onto the *in vitro* protein coated membrane. Glucose and insulin diffusion rates were significantly lower in both pre-coated and non-coated implants, indicating that the protein coating could be a reason for the drop in permeability *in vivo*.

Islet sheet. The islet sheet™ (**Figure 2D**) is a prototype of a multi-layered islet encapsulation device constructed from alginate. The islets are inserted in a flat sheet sealed on both sides by acellular layers of purified alginate, which serve as immunoisolation layers, without any polymer reinforcement. The islet sheet thickness is maintained as small as possible (~250 μm) [115]. Like other intraperitoneally implanted encapsulation devices, this device relies primarily on passive

diffusion for nutrient, oxygen, insulin, and metabolites transport. One study demonstrated fasting normoglycemia in a pancreatectomized dog, implanted with sheets containing encapsulated allogeneic islets implanted in the omentum for 84 days post transplantation [115]. A previous study using hollow fiber devices [107] has demonstrated that surface irregularities can trigger fibroblast attachment and consequently lead to fibrosis and implant failure. Thus, it is expected that a planar device like the islet sheet™ would demonstrate minimal fibroblast activation and fibrosis owing to its smooth and continuous outer membrane layers.

Vascularized devices. The idea of devising 'vascularized' devices to improve islet nutrient and oxygen supply, thus promoting improved insulin secretion and increasing the overall efficiency of encapsulation devices, was developed during the last decade. To build such a device researchers first implant a 'scaffold', usually a few days or weeks before islet implantation. This 'scaffold' promotes neovascularization, the sprouting of new capillaries around, and in some instances into the implant, and helps attenuate hypoxic injury to the transplanted islets, especially during the first week immediately after transplantation when they are most vulnerable to such injury. Studies evaluating the utility of polytetrafluoroethylene (PTFE) in the formulation of a scaffold for use in islet encapsulation either alone [128], or with a coating of acidic fibroblast growth factor (a-FGF) [77] (implanted into recipient animals one month before islet seeding) concluded that, in both instances, vascularization was induced after sub-epidermal or intraperitoneal implantation. Islets were seeded within the device 4 weeks later. In successful experiments, the diabetic recipients demonstrated sustained normoglycemia for up to 6 months. When the device was retrieved at the end of the study, over half of the encapsulated islets stained positive for insulin.

Juang *et al*. compared the efficacy and performance of thin fibrils made of either polyvinyl alcohol (PVA) or polyglycolic acid (PGA) woven into flat sheets, within which free or encapsulated allogeneic islets were seeded, implanted at one of three sites: renal subcapsular, intraperitoneal, and subcutaneous [123]. These studies demonstrated that PGA polymer sheets transplanted subcutaneously had the best results with 80% of recipients maintaining euglycemia for 3 months after implantation. Upon retrieval, successfully implanted devices also demonstrated numerous intact islets and implant neovascularization.

Theracyte. The Theracyte device (Baxter Healthcare, Round Lake, Ill., USA) is a durable and retrievable planar macroencapsulation device that consists of a bilayer polymer membrane with a 5 μm polytetrafluoroethylene (PTFE) outer layer, laminated onto a 0.45 μm inner PTFE layer [97]. A polyester mesh is attached to the 5μm PTFE layer. At one end of the device, a polyethylene port provides for access to the lumen for islet seeding (**Figure 2C**). This device supports neovascularization via the outer membranes while containing the engrafted cells within cell-impermeable inner membranes [97], an arrangement that avoids contact with the host immune cells and vasculature. This was demonstrated by Rafael *et al*. who observed that, during the first 4 weeks after implantation, the device constitutes a significant diffusion barrier with significantly lower peak glucose levels, longer times-to-peak, and smaller areas under the curve, but at 3 months, the exchange between the lumen of devices and the blood stream improved significantly.

Subsequent studies comparing Theracyte™ devices that were pre-implanted 3 months before islet seeding with devices implanted concurrently with the islets demonstrated a greater volume of viable islets, lower incidence of fibrosis, and higher proportion of insulin-positive β-cells 2 weeks post-implantation [129, 130]. One study has reported that human islets or islet-like cell clusters (ICCs) encapsulated within Theracyte™ devices remain viable and functional even after prolonged cryopreservation [131]. Allogeneic rodent islets [132] and human fetal ICCs [93], encapsulated within Theracyte™ devices and transplanted into allosensitized rats and immunodeficient mice respectively, have proved to be viable and functional for up to 6 months post transplantation.

Microencapsulation. In the case of microencapsulation, islets are immobilized inside microspheres of alginate, agarose gel, or another biocompatible material and implanted into the recipient. Compared to planar and tubular macro capsules, alginate microcapsules (**Figure 2E**) are mechanically more stable, have a better surface area to volume ratio, and a superior immunologic profile [133, 134], are simple to construct, and provide incredible flexibility to manipulate key parameters including wall thickness and pore size. Consequently, they remain the most commonly employed method [134-136] in islet encapsulation. Since these capsules can be mass produced by "encapsulators" using commercially developed air-jet driven droplet technology [137, 138], they are cost-effective, and can be standardized for clinical use. They have been safely tested in numerous small and large animal trials and would thus be safe for widespread application in clinical islet transplantation. Alginate microencapsulation has demonstrated graft protection from host immune attack and prolonged islet survival without immunosuppression in studies conducted in small [139] and large diabetic animal models [93].

Several stimuli-responsive hydrogels are among the most commonly used synthetic agents in encapsulation and tissue engineering including poly (vinyl methyl ether), poly acrylamide gels, poly vinyl alcohol, polyphosphazene, and other derivatives [140]. The most significant drawback with using synthetic scaffolds is the significant potential of a host inflammatory response being elicited against the foreign material leading to fibrosis and loss of the encapsulated cells. All synthetic constituents would also be required to be manufactured and purified using materials and methods that would have to be non-toxic, while also not subjecting the cells to excessive mechanical or chemical stress. They are typically also modified to be able to interact with the environment and gradually degrade under physiologic conditions. Unlike synthetic materials, naturally occurring hydrogels such as gelatin, fibrin, agarose, hyaluronate, chitosan, and alginate [141, 142] are less likely to induce a fibrotic or inflammatory host response and are hence preferred for use in microencapsulation. Collagen is the most common naturally occurring hydrogel used in the formulation of microencapsulation devices. However, naturally occurring hydrogels also have their disadvantages, lower tensile strength, high cost and greater inter-batch variations, thus impeding standardization of the manufacturing process [141].

[Insert Figure 3 here]

3.3 Current advances in encapsulation technology

Although encapsulation should theoretically ensure immune isolation and free oxygen and nutrient diffusion across the matrix (**Figure 3**), in reality, graft rejection and necrosis have been observed [143, 144], which have led researchers to believe that hypoxic injury and apoptosis is

to blame. Some researchers have addressed these issues by employing layer-by-layer coating (**Figure 2F**), which involves the generation of a complex of nanocoatings by adding additional layers surrounding the islets to achieve adequate immunoisolation while preserving optimum diffusion parameters.

Nanoencapsulation. Nanoencapsulation is achieved by creating an efficient and biocompatible nanoscale immunoisolation layer adjacent to the cell surface (**Figure 2G**), thus eliminating diffusion barriers, while also allowing for implantation of the encapsulated islets into sites normally suitable only for non-encapsulated islets [145]. This technique confers several advantages over conventional micro- and macroencapsulation techniques especially enhanced glucose response time, superior nutrient access, the possibility of 'tuning' permeability by controlling layer thickness and composition without a significant increase in the size of the islet or the protective envelope. In addition, enhanced biocompatibility and islet survival could also be achieved by incorporation of immunosuppressive drugs into the capsule for sustained release into the tissue interstitium.

Several methods of nanoencapsulation have been used in islet encapsulation, of which 'PEG'ylation, or nanocoating islets with polyethylene glycol (PEG) is the most popular [146]. Exposure of PEG to ultraviolet or visible light triggers cross-linking which results in the formation of a 'nanocapsule' around the islet with minimal tissue damage [53]. However, PEG is less biocompatible than many hydrogels currently being studied for use in islet encapsulation and it cannot effectively protect the encapsulated cells against cytokine attack [147]. Layer-by-layer (LBL) nanoencapsulation with multiple layers of polyelectrolyte [148], polyvinyl alcohol

conjugated to a single layer of PEG-phospholipid [149], or the incorporation of biological factors like FGF-1 [150], anti-coagulants [151], or anti-inflammatory molecules [152-156] are being researched to address this issue. LBL encapsulation has also been attempted in novel areas such as delayed-release pharmacotherapy, antioxidants, and even oxygen-generating biomaterials (PolyDiMethylSiloxane (PDMS)-encapsulated solid calcium peroxide) [157] in an attempt to address encapsulation induced hypoxia. Alginate–chitosan nanolayers [158], polylysine/polyglutamic acid membranes [159], poly (l-lysine) - g-poly (ethylene glycol) (biotin) (PPB) and streptavidin (SA) [160], complement receptor 1 and heparin [161], and PEG–lipid and PVA [149, 162] are some of the many biomaterials currently being studied for use in islet nanoencapsulation.

3.4 Stem cells

Stem cells are an attractive alternative to human islets, as the limited availability of transplant-worthy human cadaveric donors, the need for multiple donors per recipient, and low islet yields remain an obstacle to the widespread application of human islet allotransplantation in achieving a long term cure for T1D. Viable insulin-producing β-cells can be sourced from various kinds of stem cells (human embryonic, induced pluripotent, mesenchymal etc.) for transplantation. Although several *in vivo* studies have been conducted in this field, the results have not been consistently encouraging.

Viacyte LLC (San Diego, CA), a pioneer in the area of encapsulated stem cell-related transplants, has demonstrated the feasibility of encapsulating human embryonic stem cells (hESC) within the Theracyte™ device and efficiently directing the encapsulated cells down a

pancreatic endocrine lineage, despite not having any direct contact with the host environment. In one study, diabetic host mice transplanted with these cells encapsulated within the Theracyte™ device demonstrated euglycemia within three months after receiving the aforementioned device [163]. In another study, although initial glucose-stimulated insulin responses and plasma c-peptide levels remained low 12 weeks after transplantation, after 5 months, both parameters improved remarkably, suggesting that differentiation continued post encapsulation [93]. It has even been demonstrated that human insulin expressing cells encapsulated using the Theracyte device can be safely cryopreserved, then thawed and still retain their function [131]. However, such positive outcomes were not always observed [164]; there were instances of devices becoming walled off within fibrotic tissue and failing to stain positive for endocrine cells after retrieval. It has been demonstrated that the stiffness of the alginate substrate can affect stem cell differentiation.

Candiello *et al*. demonstrated that hESC gene expression was sensitive to changes in the stiffness of the alginate hydrogel substrate used. Endodermal gene expression demonstrated the greatest sensitivity to changes in substrate stiffness and could be manipulated in conjunction with chemical signals to guide stem cell lineage fates toward endodermal lines [165]. Alternatively, mesenchymal stem cells derived from human amnion have demonstrated the ability to transform into functional islet-like clusters which, when encapsulated in polyurethane-polyvinyl pyrrolidone or alginate microcapsules and transplanted into diabetic mice, resulted in a return to normoglycemia two weeks post transplantation. The changes were sustained until approximately 30 days after transplantation [166, 167].

Davis *et al*. demonstrated that silk-based encapsulation devices are versatile, provide an excellent encapsulation milieu for islets, and maintain islet viability and function *in vitro*. When murine islets were co-encapsulated with mesenchymal stem cells (MSCs) and extra cellular matrix (ECM) proteins within these devices, graft function and survival improved. If these results could be reproduced in small and large animal *in vivo* studies, the device could potentially represent a breakthrough in the search for a cure for T1D [168].

Another study using adipose-derived stem cells (ADSCs) reported that ICAs derived from ADSCs expressed pancreatic endocrine hormones, co-expressed insulin and somatostatin (similar to fetal pancreatic cells), and demonstrated human C-peptide secretion in response to *in vitro* glucose stimulation in a dose-dependent manner. When these ICAs were allowed to mature, encapsulated within biocompatible Polyurethane-poly vinyl pyrrolidone-Interpenetrating network (PU-PVP-IPN) microcapsules and transplanted into the peritoneal cavity of STZ-induced diabetic mice, a sustained lowering of blood glucose levels was noted within 3 weeks of transplantation. These results were sustained up to two months after surgery [169].

Mason *et al*. demonstrated that dissociated embryonic pancreatic precursor cells photoencapsulated and cultured within a synthetic PEG hydrogel selectively differentiated into insulin-secreting β-cells. The results of this study seem to suggest that embryonic pancreatic precursor cells could be exposed to specific chemical environments to encourage targeted cell proliferation and differentiation to generate a population of primarily glucose responsive β-cells [170].

Induced pluripotent stem cells (iPSCs) are another important source of stem cells that are being studied for use in islet transplantation. These cells have demonstrated the ability to differentiate into functional β-cells, *in vitro*, as demonstrated by a partial C-peptide release response after glucose stimulation [171-173]. Furthermore, iPSCs derived from mice and rhesus monkeys have demonstrated the ability to differentiate into glucose–responsive insulin positive cells with a complete reversal of experimentally induced hyperglycemia after transplantation into a mouse model of diabetes (type 1 and type 2) [174]. In addition to all the aforementioned cell types, pancreatic epithelial cells, ductal cells, and even α-cells have been demonstrated to be able to differentiate into β-cells under appropriate conditions [174].

It is still a matter of concern that no research group has achieved the vital breakthrough of sustained insulin independence using β-cells generated from stem cells. New research developments in the field of stem cell differentiation are being reported and will hopefully improve upon the method pioneered by Blyszczuk [175]. Even if researchers achieved controlled stem cell differentiation into functional β-cells with long-term insulin independence after transplantation into non-human diabetic primates, diabetic human subjects would not be able to benefit from these achievements immediately. Similar, reproducible results from multi-center, multidisciplinary, randomized, controlled, clinical trials would be required to translate their findings into an established treatment modality for patients with T1D. Although the protocols and standard operating procedures (SOPs) for the isolation, proliferation, in-vitro culture, differentiation, and maturation of stem cells derived from human embryos and induced pluripotent stem cells have been established, tested, and standardized, several significant milestones need to be reached before studies can be commenced in diabetic human subjects.

The two principal issues of importance are the inherent ability of hESC/iPSCs to proliferate rapidly and in an unpredictable manner and undergo malignant transformation and the even more worrisome risk that the implanted stem cells could be recognized as foreign cells by the host immune system and subsequently attacked and destroyed, leading to graft failure and a relapse into the diabetic state. However, if hESC or iPSC-derived insulin producing cells are enclosed in a protective, bioinert, biocompatible layer or capsule and transplanted to an appropriate location, concerns about tumor formation and immune attack can be alleviated, and adequate insulin secretion to enable the maintenance of euglycemia can be achieved [176].

4. Conclusions

Pancreas cell encapsulation is a rapidly expanding field that attempts to challenge conventionally accepted treatment paradigms and revolutionize the field of islet transplantation [177]. Stem cell therapy has established itself as a rapidly expanding and potentially limitless source of β-cells to arrive at a cure for T1D, but the issue of recognition of these foreign cells by the host immune system and subsequent destruction via either a humoral or cellular immune response remains unaddressed. Although new developments in the field of biomaterial encapsulation provide several solutions to eliminate the need for toxic immunosuppressive therapy while promoting implant engraftment, several challenges still remain [178-181]. Improvements in graft viability, encapsulation techniques, biomaterial manufacturing, and purification procedures, identification of the safest, most reliable and scalable tissue source, and refinement of islet and stem cell isolation and culture techniques are vital to translate bench research into successful clinical islet transplantation with a prolonged period of insulin independence.

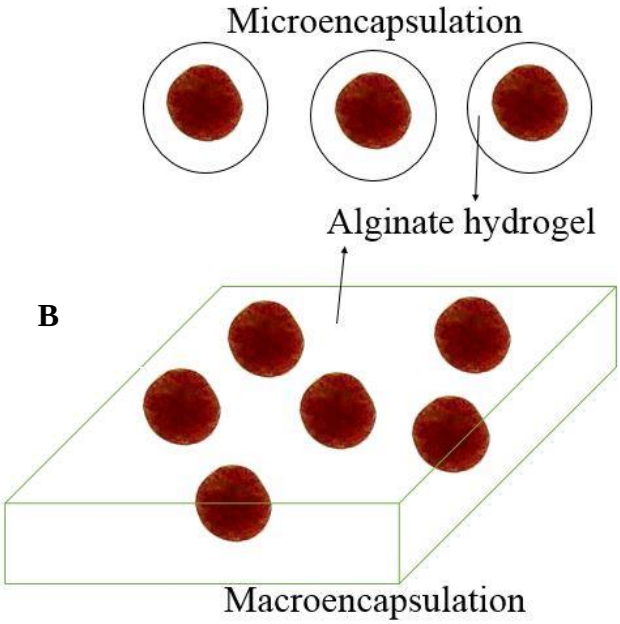

Figure 1. Types of encapsulation. Two commonly used types of cell encapsulation in alginate hydrogels: microencapsulation (**A**) and macroencapsulation (**B**)

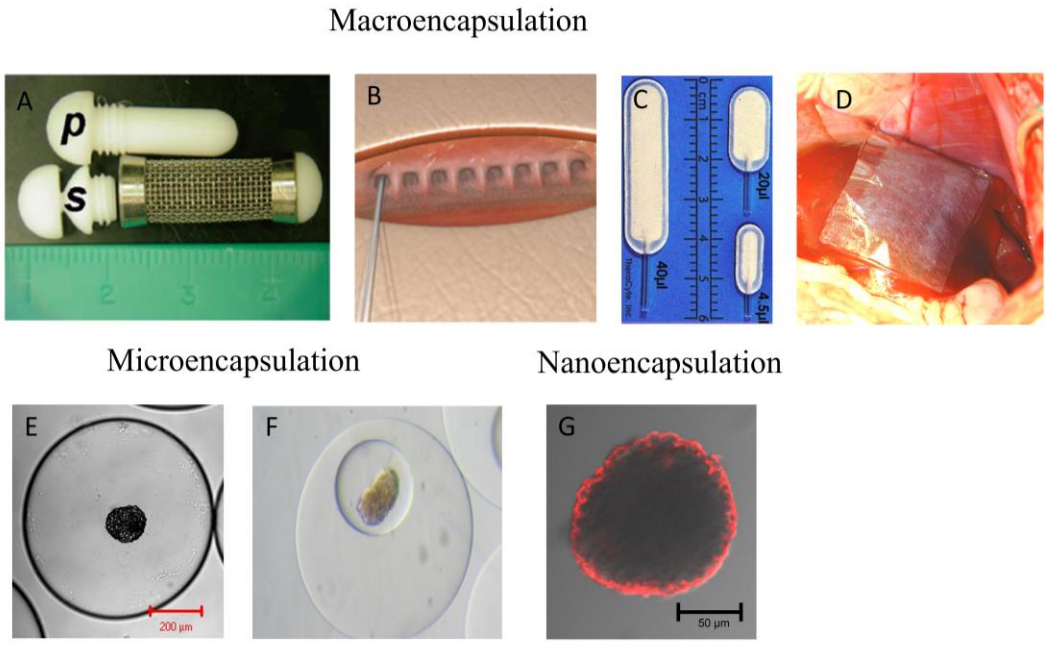

Figure 2. Variety of encapsulation devices currently in use. Biocompatible implantable device developed at the National Autonomous University of Mexico (Mexico D.F., Mexico) [124, 125] (**A**). Sernova Cell Pouch System™ [182] (**B**). Size comparison of various theracyte models [132] (**C**). Alginate sheet transplanted onto the liver [183] (**D**). Single encapsulated islet (**E**). Double encapsulated islet (**F**) (de Vos P, personal communication). Nanoencapsulated islet (**G**) (Ricordi C, personal communication).

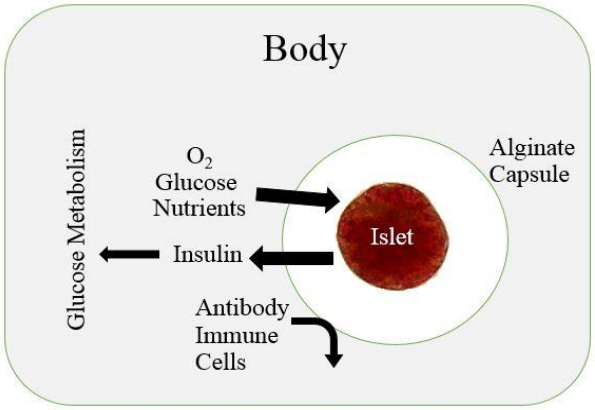

Figure 3: Schematic figure of a bioencapsulated islet. A schematic demonstrating the advantages of alginate microencapsulation. The capsule acts as an immunoisolation device which restricts the entry of immune cells and antibodies while allowing for the passive diffusion of nutrients, oxygen, and glucose into the capsules and insulin out of the capsules into the body. Encapsulation design by Islet Science, LLC. Picture provided by Dr. Jonathan RT Lakey, UCI.

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