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ASAIO Journal 2021 Review Article

The Current Status of Bioartificial Pancreas Devices

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Type 1 diabetes mellitus is a common and highly morbid disease for which there is no cure. Treatment primarily involves exogenous insulin administration, and, under specific circumstances, islet or pancreas transplantation. However, insulin replacement alone fails to replicate the endocrine function of the pancreas and does not provide durable euglycemia. In addition, transplantation requires lifelong use of immunosuppressive medications, which has deleterious side effects, is expensive, and is inappropriate for use in adolescents. A bioartificial pancreas that provides total endocrine pancreatic function without immunosuppression is a potential therapy for treatment of type 1 diabetes. Numerous models are in development and take different approaches to cell source, encapsulation method, and device implantation location. We review current therapies for type 1 diabetes mellitus, the requirements for a bioartificial pancreas, and quantitatively compare device function. *ASAIO Journal* 2021; 67:370–381.

Key Words: bioartificial pancreas, beta cell, islets of Langerhans, type 1 diabetes mellitus, encapsulation

Type 1 diabetes mellitus (T1DM) is a common and difficult to treat disease associated with high morbidity and early mortality.[1] The condition is caused by autoimmune destruction of insulin-producing β cells within the pancreas, resulting in dysregulation of blood glucose levels. The events triggering β-cell death are poorly understood but are thought to be a combination of genetic and environmental insults. There are 1.25 million people in the United States living with T1DM, and greater than 20 million people worldwide.[2] By 2020, it is estimated that one out of 300 adolescents living in the United States will be diagnosed with T1DM.[3]

The primary treatment of T1DM is exogenous insulin administration. Although insulin therapy prevents early mortality, its use does not guarantee euglycemia. Long-term fluctuations in blood glucose levels despite insulin administration ultimately results in multiorgan dysfunction.[4] Frequent fluctuations in blood glucose lead to the complications of diabetes and resulting organ failure. Obtaining physiologic control of blood glucose would allow type 1 diabetic patients to have an excellent quality of life free from multiple hospitalizations, end-stage organ failure, and early mortality.[5]

The bioartificial pancreas (BAP) is a solution that could provide the tight metabolic control needed to manage type 1 diabetes.[6] Multiple devices are currently in preclinical or early clinical studies.[7–9] although numerous reviews have discussed encapsulation strategies and insulin-producing cell sources,[10,11] none have offered a quantitative comparison of device function. We give an overview of the currently available treatments for type 1 diabetes, the current bioartificial pancreas devices, and provide a quantitative comparison between devices that will inform future BAP design.

Part I: Current Therapies for Type 1 Diabetes Mellitus

Exogenous Insulin Therapy

Exogenous insulin therapy is the primary treatment for T1DM.[12] However, the complex interplay between multiple endocrine hormones that controls glucose homeostasis cannot be reproduced by insulin therapy alone. As such, patients managed with exogenous insulin therapy experience frequent episodes of acute hypoglycemia and hyperglycemia.[13] The resulting blood sugar dysregulation can lead to serious complications, such as diabetic ketoacidosis (DKA), and death.[14] In the long term, hyperglycemia causes microvascular disease resulting in retinopathy, peripheral vascular disease, end-stage renal disease, and cardiovascular disease, which are the major causes of morbidity and mortality of T1DM.[15] Technology that enables maintenance of strict glucose homeostasis is necessary.

An evolving method for treating patients with T1DM is infusion of physiologic basal levels of insulin and bolus dosing for food intake *via* an insulin pump paired with a continuous glucose monitor that calculates insulin requirements according to blood glucose levels.[16] Although insulin pumps have resulted in increased glycemic control, there are several problems with insulin pumps, including mechanical and electrical malfunctions of the pump and infusion system, cutaneous infections and scarring, and imperfect blood glucose regulation, potentially resulting in immediately life threatening hyper- or hypoglycemia.[17,18] One prospective study reports an adverse event rate of 40 per 100 person-years, with the most common adverse events being hyperglycemia and DKA requiring hospitalization.[19] Additionally, this technology is expensive and requires a high level of patient education and compliance.[20] When insulin pumps are not an adequate or attainable solution for diabetes management, pancreas transplantation is the treatment of choice.

Pancreas Transplantation

There are many problems preventing widespread usage of pancreas transplantation for the treatment of T1DM. Organ scarcity, complexity of the operation, posttransplant morbidity,

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Submitted for consideration February 2020; accepted for publication in revised form June 2020.

Disclosure: The authors have no conflicts of interest to report.

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DOI: 10.1097/MAT.0000000000001252

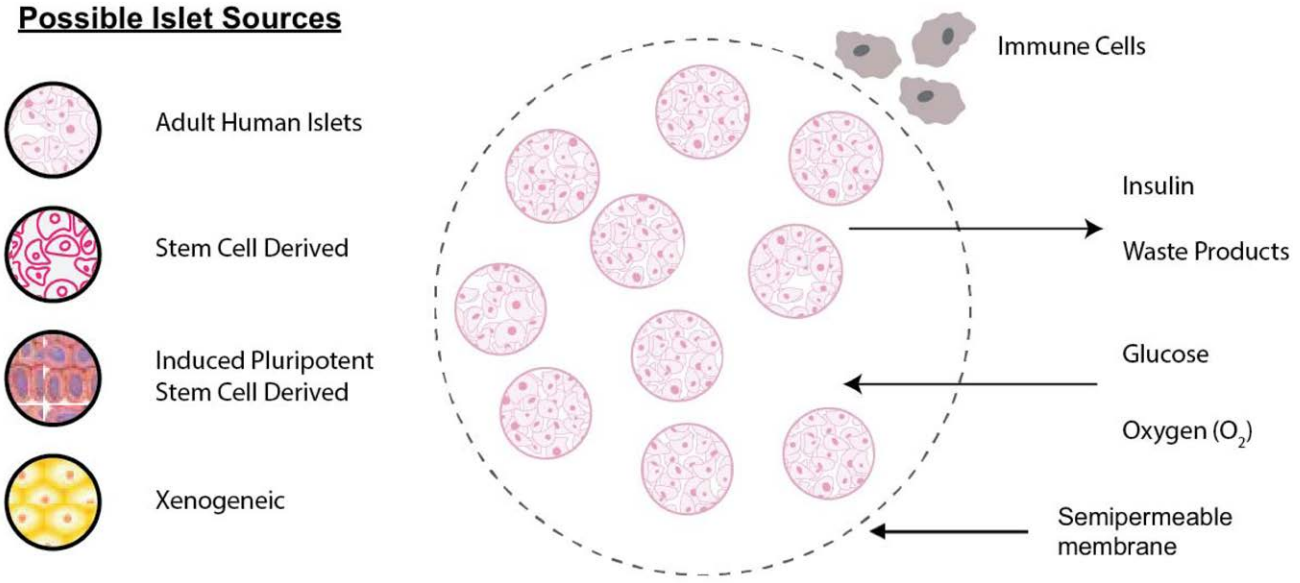

Figure 1. Bioartificial pancreas concept: Islets or islet-like cells surrounded by a semipermeable, immune-protective barrier (dotted line). Small molecules, including oxygen, glucose, and insulin, can freely cross the barrier while large immune system components are prevented from infiltrating the barrier. Potential islet sources include human adult islets, stem cell–derived beta-like cells, beta cells derived from induced-pluripotent stem cells, and xenogeneic sources. full color online

and immunosuppression are significant barriers to pancreas transplantation.[21] Lifelong immunosuppression causes direct toxicity to the heart, kidneys, and gastrointestinal tract.[22] The resulting immune dysfunction increases the risk of infection and malignancy,[23] rendering immunosuppression dangerous for any patient and specifically precludes their use in the pediatric population. Furthermore, at greater than 10 years post-transplant graft function declines to less than 50%.[24]

Clinical Islet Transplantation

Percutaneous clinical islet cell transplantation (CIT) is an endovascular procedure involving injection of purified islets into the portal vein, which then migrate to and ultimately reside in the liver.[25] Since the first islet cell transplant was performed in 1999, more than 1,500 patients have undergone the procedure.[26] In a multinational phase III, clinical trial involving 48 patients with severe hypoglycemic unawareness, 87.5% and 71% of patients maintained near-euglycemia at the one and two year mark, respectively.[27]

Although islet transplantation has achieved success in obtaining glycemic control, there are major drawbacks to the procedure. The instant blood mediated inflammatory reaction (IBMIR) is characterized by the host's innate immune system attacking the newly implanted cells, which drastically reduces the number of transplanted islets.[28] Antiinflammatory agents, such as anti-TNF alpha biologics and IL-1 antagonists, have been used in an effort to dampen this response with inconsistent success.[29,30] Equally detrimental is the need for lifelong immunosuppression after islet cell transplantation.[22]

The medical and scientific community is developing the bioartificial pancreas, with the goal of delivering the same therapeutic benefit of clinical islet transplantation. Bioartificial pancreas devices offer distinct advantages over percutaneous islet cell transplantation. The cells are protected within an immune-protective environment, which eliminates both the IBMIR response and the immunosuppressive requirements.[6] The protective barriers of a bioartificial pancreas device are thought to promote islet longevity and result in long-term endocrine function.[11]

Part II: Engineering a Bioartificial Pancreas

The bioartificial pancreas comprises insulin-producing cells surrounded by semipermeable encapsulating membranes[31] (**Figure 1**). The design of a bioartificial pancreas is informed by the architecture of the human pancreas and islet physiology, as mimicking the natural islet environment in a BAP device will increase the chance of successful long-term device function. After implantation, the pancreatic graft requires adequate oxygenation, exchange of stimulants and products, and protection from immune rejection,[32] which can be achieved by optimizing the encapsulation strategy, cell source, and implantation location.[33]

Islet Physiology

Scattered throughout the pancreas are 70–250 μm-diameter spheroid structures called the islets of Langerhans[34] (**Figure 2**). The islets are composed of multiple cell types, including beta cells that produce insulin, alpha cells that produce glucagon, delta cells that produce somatostatin, and PP cells which produce polypeptide protein. Endocrine cells only comprise 1–2% of the pancreas by volume,[35] but use between 5 and 20% of the flow depending on metabolic needs[36] (**Figure 3**). Each islet is fed by two or three afferent arterioles, and, within the islets, the arterioles become highly fenestrated.[37] The complexity of islet blood supply suggests a highly tunable system in which the amount of blood flow is modulated at the level of the feeding arteriole based on metabolic needs. During the process of islet isolation, the blood supply to islets becomes deranged,[38] undoubtedly affecting their functionality in medical devices. Restoring islet nutrient supply is central to long-term device function and success in treating T1DM.

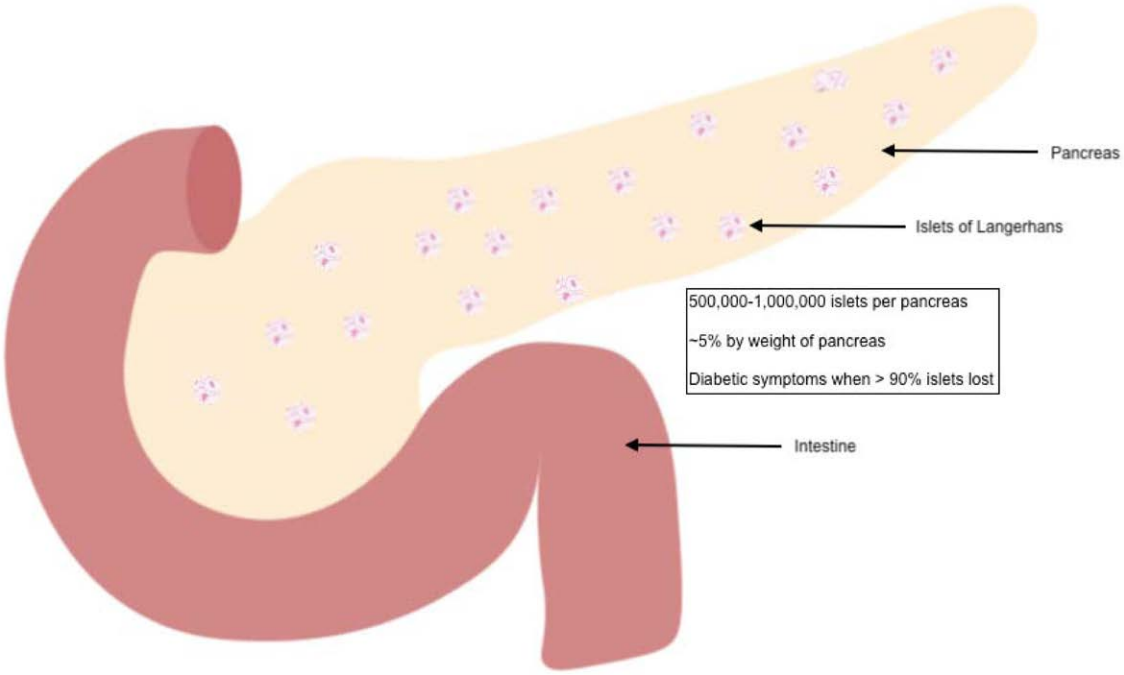

Figure 2. Pancreas anatomy: The elongate pancreas abuts the small intestine. Islets of Langerhans, which number roughly 500,000–1 million islets in a human, are distributed evenly throughout the pancreas. full color online

Cell Sources for the Implanted Bioartificial Pancreas

Because the number of islets within a human pancreas ranges from several hundred thousand to millions,[37] there is a need for alternative sources of islets for a bioartificial pancreas. Organs scarcity and the costly specialized centers necessary for islet isolation and purification preclude adult human pancreases from being a source for islets.[39] Alternate sources of islets and islet-like cells ease reliance on cadaveric islets, which are scarce and potentially compromised depending on the donor's comorbidities and cause of death. Among the contenders are beta-like cells derived from human pluripotent stem cells such as embryonic and induced-pluripotent stem cells, human hepatocyte-derived islet-like cells, and xenogeneic sources.[40]

Two promising beta-cell sources are differentiation from human embryonic stem cells (hESC) and human induced-pluripotent stem cells (hiPSC).[41,42] The differentiation of pancreatic endocrine cells from embryonic stem cells is a multistep pathway tightly regulated by the introduction of signaling molecules[43] (**Figure 4**). Agulnick *et al*.[44] demonstrated success at differentiating highly enriched pancreatic endocrine cells in a seven-step process that has shown efficacy in reversing diabetes in a mouse model. Human pluripotent stem cells are particularly appealing as they can be derived from human somatic cells, such as skin fibroblasts.[45] Early studies demonstrate that beta-like cells derived from hiPSC have reversed hyperglycemia in a diabetic mouse model.[46] Although successes have been achieved, there are still significant barriers to human implementation, including scalability, immune protection of the cells, exclusion of polyhormonal cell types, and maximizing phenotypically normal beta-like cells that response to glucose stimulation.[47]

An alternative cell source is derivation from other human somatic cells, such as hepatocytes. Human hepatocytes provide an opportunity to create islet-like cells given the presence of the GLUT two glucose transporter and the glucose phosphorylating protein, glucokinase. These two elements are essential for sensing changes in blood glucose and the secretion of insulin granules. Lawandi *et al*.[48] demonstrated the use of Melligen cells, or human hepatocyte-derived islet-like cells, in the maintenance of normoglycemia in the diabetic mouse model. The significant limitation toward human use of Melligen cells is that the cells continue to proliferate, risking tumorigenesis and eventual hypoglycemia. Despite these risks, Melligen cells offer tremendous potential as a beta-cell source, and Pharmacyte, Inc., is developing encapsulating technology using hepatocyte-derived cells.

Ultimately, the use of a renewable cell source or islets from xenogeneic islet sources is essential to making the bioartificial pancreas device a reality for patients with T1DM.

Islet Encapsulation Strategies: Macroencapsulation, Microencapsulation, and Nanoencapsulation

Encapsulation strategies employing durable selectively permeable membranes that allow free exchange of nutrients and exclude immunocytes and antibodies are central to achieving the requirements for a bioartificial pancreas. The three encapsulation strategies used in BAP devices are macroencapsulation, microencapsulation, and nanoencapsulation[49] (**Figure 5**). Macroencapsulation devices are several centimeters in diameter and are made of hollow fibers, flat sheets, or disks, and they contain a central chamber which houses islets. It is essential that macroencapsulated devices are well-perfused with little internal dead space, and that fibroblastic growth on the device is minimized to allow efficient transfer of substances.[50] The primary constraint on macroencapsulated devices is achieving the ideal diffusion distance between oxygen source

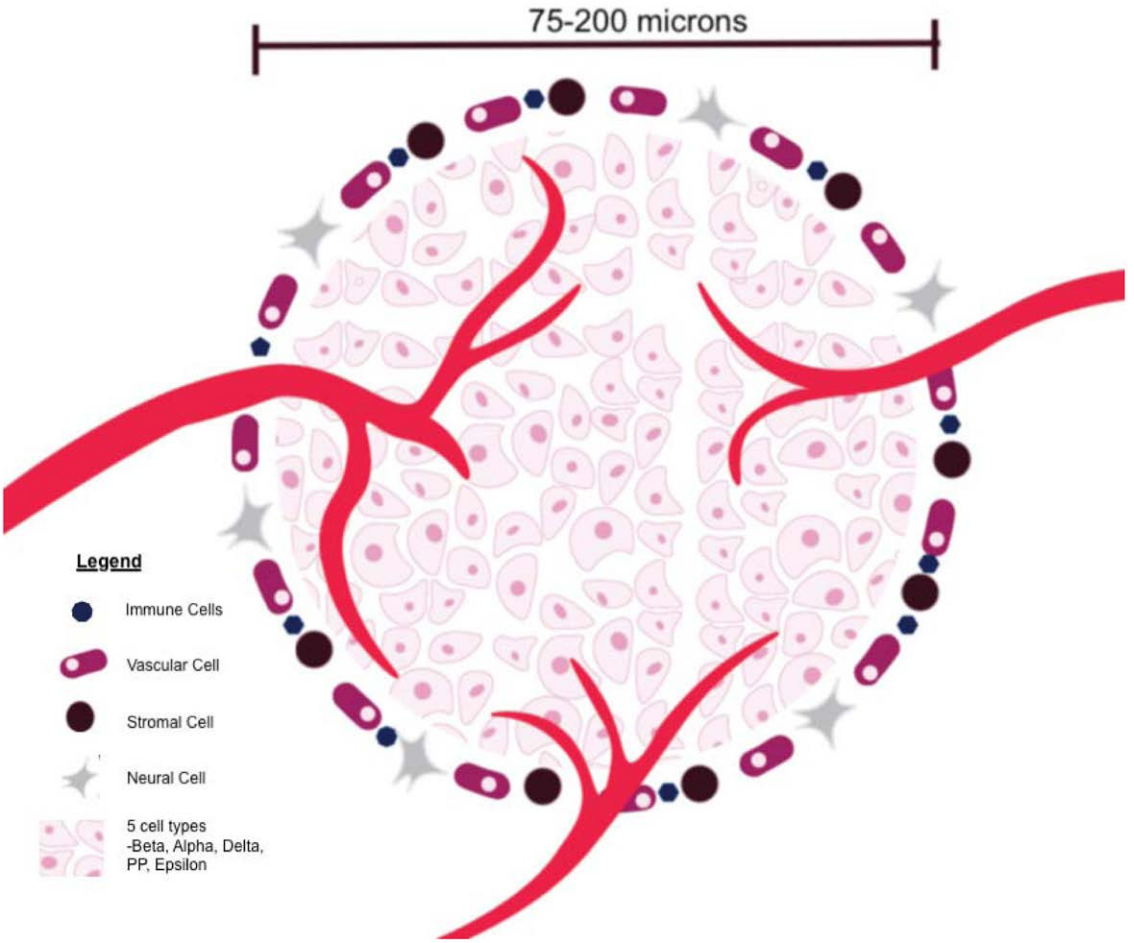

Figure 3. Islet of Langerhans physiology: Each islet is approximately 75–200 microns in diameter and is surrounded by multiple different cell types, including immune cells, vascular cells, stromal cells, and neural cells. Each islet is supplied by two to three arterioles, which branch into capillaries within the islet, supplying a rich vascular inflow. full color online

and islet, which is approximately 200 μm.[51] Multiple macroencapsulated devices are in advanced preclinical or early phase human clinical trials[8,44,52,53] (**Table 1**).

Microencapsulation techniques involve individually encapsulating islets with micrometer thick layers of biocompatible porous materials, such as hydrogels.[54] Coated islets are then infused into the body, most commonly into the intraperitoneal cavity. The primary constraints on microencapsulated islets are that exchange of substances is governed by diffusion and local concentration gradients of insulin and glucose and the immune response to the pancreatic graft. One of the limiting concepts surrounding islet encapsulation is relative hypoxia and central necrosis within the islet if diffusion distances are too great.[55] Microencapsulated islets have shown efficacy in small animal models but have shown limited efficacy in nonhuman primates.[56–58] The most significant study published demonstrated the effectiveness of alginate microencapsulated islets in streptozotocin-induced diabetic nonhuman primates with immunosuppression.[59] Normoglycemia was achieved immediately following transplant, and insulin independence was maintained for three to four days. Plasma C-peptide levels were detectable until postoperative day 10 when they rapidly

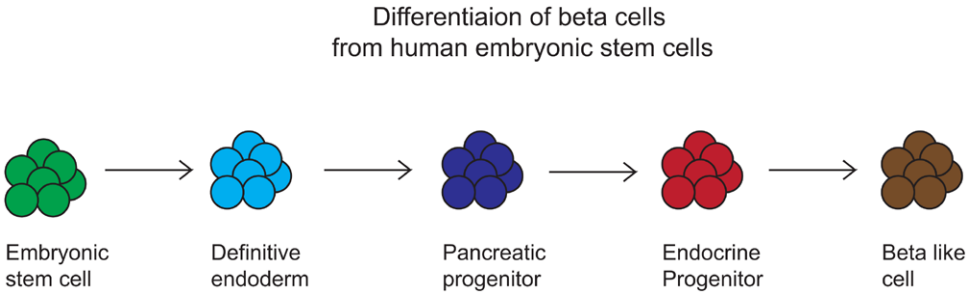

Figure 4. Beta-cell differentiation: The differentiation of beta-like cells from human ESC is a four-step process. The resulting beta-like cell mass is immature requires further refinements to adequately respond to glucose stimulation. ESC, embryonic stem cells. full color online

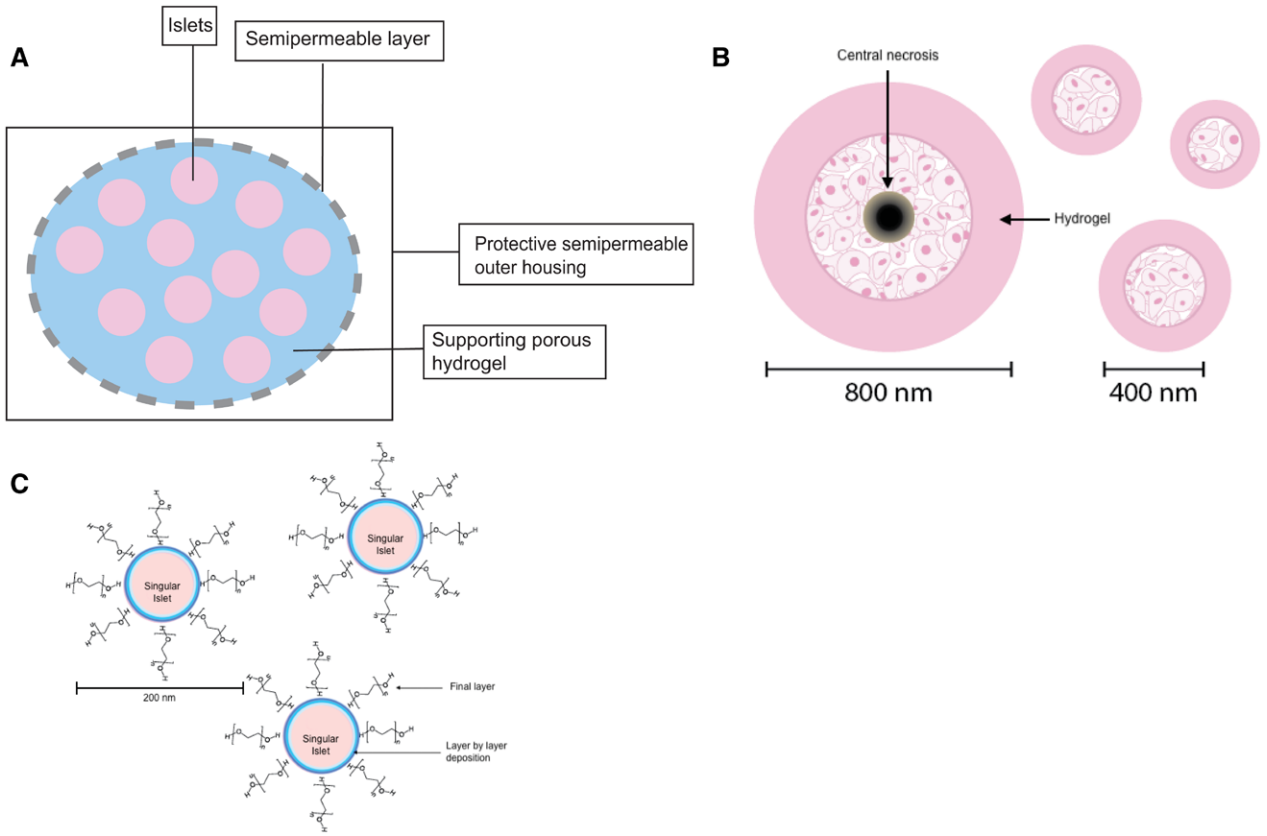

Figure 5. (A) Macroencapsulation: In this encapsulating strategy, a group of islets or islet-like cells are suspended within a supportive, porous scaffold, and encapsulated within a semipermeable layer and a protective outer housing. (B) Microencapsulation: In this encapsulating strategy, an islet or islet-like cell is coated with micrometer thick, porous, biocompatible materials such as a hydrogel. If the coating is >800 µm, the cells in the center of the islet are deprived of oxygen, and necrosis occurs. (C) Nanoencapsulation: In this encapsulating strategy, individual islets or islet-like cells are coated with multiple layers that are nanometers thick in a process called conformal coating. full color online

diminished. While there was minimal inflammatory response, there was significant islet necrosis resulting from hypoxia.

Nanoencapsulation, or conformal coating, is similar in concept to microencapsulation but involves layer by layer deposition of nanoscale layers of biocompatible materials.[60] This approach generates a coating that is nanometers thick, the goal of which is to limit the diffusion distance to the islet within. This technique has shown success in small animal models[61] but has not been tested in primates.

Location

The combination of cells with nutrient-rich housing scaffold and barrier membranes results in a bioartificial pancreas. Regardless of whether the structure is a macro- or microstructure, the implantation location is equally important. The devices can be implanted extravascularly or intravascularly.[62] Ease of implantation, retrievability, and proximity to nutrient source are the main factors to assess when choosing an implantation location.

Extravascular implantation in subcutaneous tissues is attractive in its surgical practicality, removal, replenishment, and avoidance of vital organs.[63] Conversely, low surface area to volume ratio, poor vascularization, and diffusion constraints limit islet performance. The geometry of extravascular devices and the relative avascular location of implantation leads to poor diffusion of nutrients and insulin within the device due to large (>200 µm) diffusion distances.[51] This results in poor glucose-insulin kinetics and, ultimately, islet cell death. Additionally, subcutaneous implantation stimulates a brisk foreign body response initiated by macrophage migration to the affected area followed by macrophage fusion into foreign body giant cells, ultimately resulting in fibrosis,[8,53,64] which increases the diffusion distance. Accordingly, there is a strong effort to modify current devices to address poor vascularization for which the primary strategies are induction of neovascularization, implantation into a highly vascularized location, and direct infusion of oxygen.

The intravascular location is an alternative implantation location. Arterial blood allows efficient exchange of nutrients while preventing local accumulation of molecules that eliminate concentration gradients.[65] The most important limitations to an intravascular device are the complexity of surgery involved, the lack of retrievability, and the risk of thrombotic and hemorrhagic complications.[66–69] However, improvement in vascular surgery techniques and anticoagulation strategies using medications is reinvigorating interest in the development of an intravascular bioartificial pancreas device. Recently, Roy *et al.* described the feasibility of an intravascular implantation of a BAP developed using microelectromechanical systems technology; however, there is limited data on glucose-insulin kinetics *in vivo*.[70,71]

Table 1. Summary of Results from Device Preclinical and Clinical Testing.

Trial/ Manufacturer, y	Animal Model, n	Islet Type	IEQ/kg Body Weight Transplanted	Implant Location	Decreased Insulin Needs	Basal C-peptide Levels	Inflammatory Response	Study Duration (mo)	Findings
CIT-O7 trial (Edmonton protocol) 2016[73]	Humans, 48	Adult human islets	11,982	Liver	Yes	Physiologic	Donor-specific antibody formation	24	1. 87.5% patients achieved primary end point: HbA1c < 7% at day 365 and no SHE after day 28 2. Insulin independence was achieved by 52.1% by day 365 3. 42% remained insulin independent at 730 d
Viacyte 2015[44]	SCID/beige mice, five cohorts	hESC derived	N/A	Subcutaneous	N/A	Physiologic and supratherapeutic cohorts	N/A	6	1. Pancreatic endocrine cells derived *in vitro* function *in vivo* 2. Cryopreserved pancreatic endocrine cells function *in vivo* 3. Cells do not begin to function until 8 weeks
DRC, Brussels, Viacyte, Beta Cell Therapy Consortium 2018[71]	NSG mice, 17	hESC derived	2×10^8	Subcutaneous	N/A	Supratherapeutic	N/A	11.5	1. Sustained basal C-peptide levels over 50 wks 2. 26% of implants achieve c- peptide levels > 6ng/mL by week 20 3. Cell loss at week 50 varied between 3% and 87% between devices. Beta cell number varied between 15,000 and 600,000.
DRC Nestle macrosheet, Beta Cell Therapy Consortium 2019[52]	SCID beige mice, 58	hiPSC derived	1.2×10^8 or 6×10^8	Subcutaneous	N/A	Physiologic and supratherapeutic cohorts	N/A	4.6	1. The use of a preformed pouch was associated with increased C-peptide release from the implant 2. Increase in cell mass did not result in increases basal or stimulated C-peptide secretion
University of Mansoura, Theracyte device 2018[53]	Mongrel diabetic dog model, 6	IPCs derived from human bone-marrow MSC	5×10^6	Rectus Sheath	No	Subtherapeutic	Pericapsular fibrous capsule with cellular infiltrate that was strongly cell mediated CD3+. No CD20+ cells	18	1. Fasting euglycemia in 4/6 dogs within 8 weeks 2. Sustained subtherapeutic basal c peptide levels over a period of 6 mo 3. The % IPC increased from 3% to 22% after 6 mo *in vivo*
Beta Air 2013[72]	Streptozotocin-induced diabetic minipigs, 8	Rat islets	6,556	Subcutaneous	Yes	Physiologic	Implantation site had thin, vascularized fibrotic tissue. No evidence of serum antirat Ab. Membranes impermeable to C1q or IgG	3	1. Normoglycemia achieved for 60 d using xenogeneic rat islet 2. The membranes prevented leakage of rat antigens into the pig serum and prevented diffusion of pig IgG and C1q
Beta Air 2017[8]	Type 1 diabetic human, 4	Adult human islets	2,000-4,000	Subcutaneous	No	Subtherapeutic	Nonadherent thin fibrotic tissue surrounding the device. Increase in CD45+ cells, most of which were CD 68+ macrophages, and CD8+ cells	6	1. Fasting C peptide levels were increased for 8 weeks post transplantation 2. No HLA Ab response to islets 3. Inflammatory, fibrotic response to the device 4. Transplantation did not result in decreased insulin needs

(*Continued*)

Table 1. (*Continued*)

Trial/ Manufacturer, y	Animal Model, n	Islet Type	IEQ/kg Body Weight Transplanted	Implant Location	Decreased Insulin Needs	Basal C-peptide Levels	Inflammatory Response	Study Duration (mo)	Findings
Beta Air 2017[64]	Diabetic Rhesus macaques model, n=3	Porcine islets	20,000	Subcutaneous	Yes	Supratherapeutic	Implantation site had a thin, vascularized fibrous capsule. No CD8, CD3, CD68 rarely present	6	1. Xenogeneic transplant function without immunosuppression 2. Failure to achieve insulin independence
Pharmacyte 2015[48]	Diabetic NOD/ SCID mouse model, 16	Melligen cells	N/A	Subcutaneous	N/A	N/A	Melligen cell insulin response unaffected by cytokine exposure, Huh7 cells were nonfunctional when exposed to cytokine	1	1. Melligen cells maintained normoglycemia from day 19–27 2. Melligen cells proliferate *in vivo* carrying the risk of hypoglycemia and tumorigenesis 3. Melligen cells are resistant to cytokine destruction

Quantitated basal and stimulated C-peptide and blood glucose levels shown in Figures 7–9, when available. CIT, clinical islet cell transplantation; DRC, Diabetes Research Center; hESC, human embryonic stem cells; hiPSC, human induced pluripotent stem cells; IPCs, insulin-producing cells; MSC, mesenchymal stem cell; N/A, not applicable.

Part III: Analysis of Current Bioartificial Pancreas Devices

There are several bioartificial pancreas devices that have undergone rigorous preclinical and clinical testing including Viacyte's Encaptra device (ViaCyte, Inc., San Diego, CA), the Diabetes Research Center's (DRC) planar macro-sheets developed by Nestle Research, Lausanne, Switzerland, βO2 Technologies' βAir device (βO2 Technologies, LTD., Rosh Haayin, Israel), and Theracyte's BAP device (TheraCyte Inc., Laguna Hills, CA) (**Figure 6**). The four devices are extravascular, subcutaneous devices, and have shown efficacy in reducing insulin dependence in animal models. Comparing basic measures of device functionality such as basal and stimulated C-peptide levels, islet load, and glucose-insulin kinetics exposes the shortcomings of each design and will inform future iterations of the BAP. Details of device design and testing may be found in **Table 1**.

A bioartificial pancreas device must secrete insulin within physiologic ranges to cure T1DM. Two devices have achieved both basal and stimulated C-peptide levels within the physiologic range: Viacyte's Encaptra device using reaggregated (RA) ESC-derived insulin-producing cells (IPCs) and DRC planar sheets using iPSC-derived IPC's[44,52,71] (**Figure 7**). βAir's device used in a porcine model demonstrated normal basal C-peptide levels but stimulated values were not measured.[72] These studies demonstrate that a BAP device can secrete clinically relevant levels of C-peptide and levels comparable to those measured in the phase 3 CIT cohort.[73]

Multiple devices show either subtherapeutic or supratherapeutic levels of basal and stimulated C-peptide. Subtherapeutic C-peptide levels were seen in Theracyte's device using mesenchymal stem cell (MSC)-derived IPC's and in βAir's device using allogenic human islets in a human clinical trial[8,53,74] (**Figure 7**). The poor function of Theracyte's device may be secondary to low numbers of functional IPCs whereas βAir's device's subtherapeutic function is secondary to a low number of islets. While subtherapeutic levels of C-peptide are undesirable, more work is needed to understand the potential deleterious effects of supratherapeutic levels of C-peptide. Levels of C-peptide that are inappropriately elevated, if proportional to insulin concentrations, could have disastrous consequences *in vivo* such as unintentional hypoglycemic episodes.[75] Viacyte's Encaptra device using PEC-01 cell line showed supratherapeutic values for both basal and stimulated conditions[44] (**Figure 7**). The PEC-01 cell line is known to have pancreatic progenitor (PP) cells, which the authors conclude are responsible for the hyperactive endocrine function. The DRC planar sheets implanted in a preformed pouch also demonstrate high basal and stimulated C-peptide levels[52] (**Figure 7A** and B), which may be secondary to enhanced neovascularization and engraftment of the device, leading to faster exchange of insulin and glucose. βAir's device using porcine islets in a nonhuman primate also show elevated basal and stimulated C-peptide levels.[64] This device contained approximately two times the number of IEQ/kg BW as the CIT cohort, which could explain the increase in C-peptide levels. More work is needed to understand the consequence of supratherapeutic levels of C-peptide in large animal and human models.

Essential to developing a clinically relevant BAP is using enough islets to achieve physiologic insulin levels. In the CIT phase 3 cohort, ~11,000 IEQ/kg BW was used per patient,

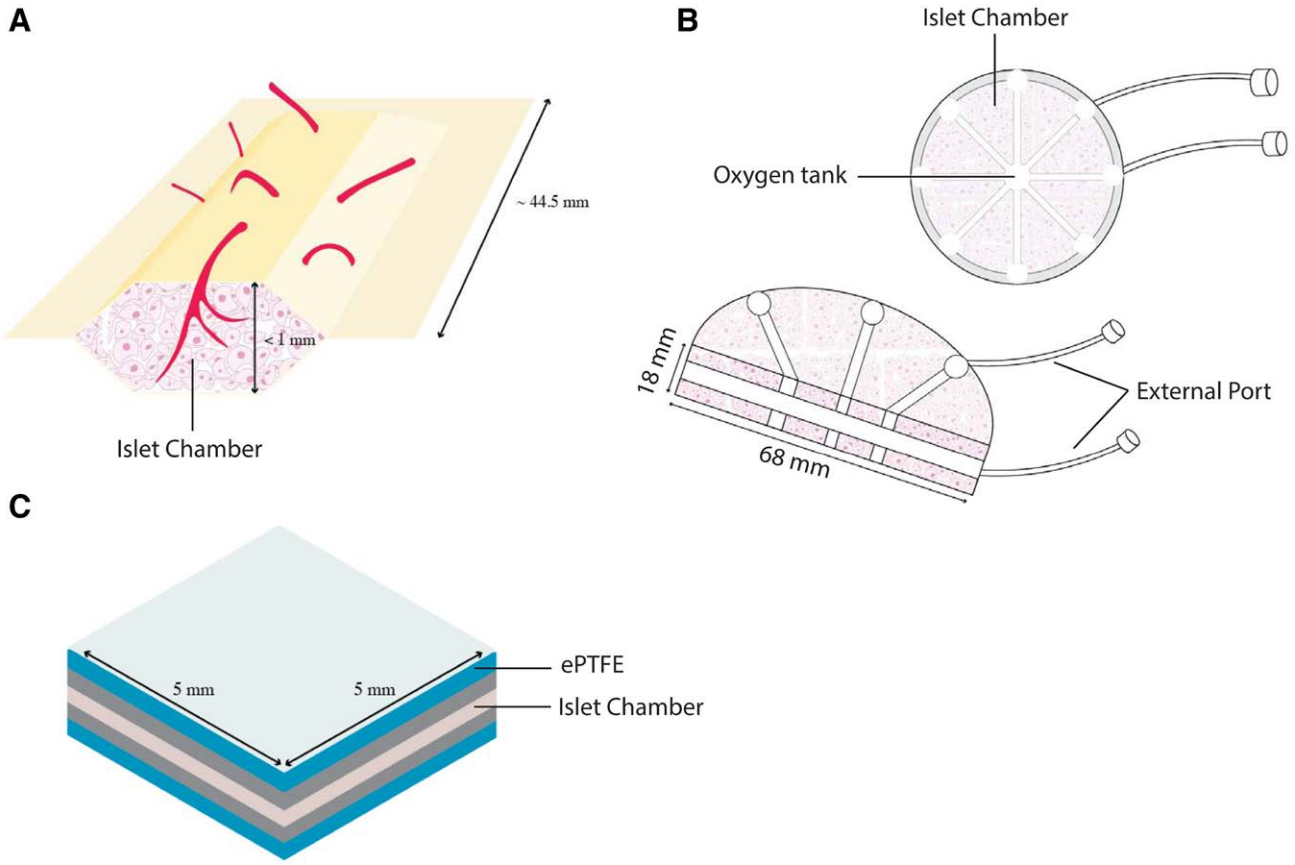

Figure 6. Bioartificial pancreas devices: (A) Viacyte's PEC-encaptra device: The PEC-encaptra device is a macroencapsulating structure that promotes neovascularization to support beta-like cells derived from hESCs. (B) Beta-Air device: The beta-air device features a central oxygen tank with two external ports that allow refilling the oxygen chamber and venting. Multiple islet chambers are supplied by the central oxygen tank with supplemental oxygen. (C) Nestle macro-sheets: Here, the islets are sandwiched between two layers of porous biocompatible materials. hESC, human embryonic stem cells; PEC, pancreatic endocrine cell. full color online

which resulted in 78% insulin independence at the two year mark.[73] We compared devices based on IEQ/kg BW implanted and found that as the number of IEQ/kg BW is increased there is diminishing return in C-peptide expression (**Figure 7**). There is tremendous variability in IEQ or IPCs per kg BW implanted between BAP devices, ranging between 1,200–6×10^8 IEQ or IPCs per kg BW. There is a linear increase in C-peptide expression with increasing IEQ/kg BW up to 20,000 IEQ/kg BW, above which there is no further increase in C-peptide secretion as IEQ/kg BW is increased. This trend suggests there is an optimal dose of IEQ/kg BW, above which a plateau is reached and increasing numbers of islets are superfluous.

To understand the maturation of stem cell–derived IPCs, we evaluated their C-peptide secretion over time (**Figure 8**). Stem cell–derived IPCs do not begin to function until eight weeks after engraftment,[44] a finding which has been corroborated by multiple groups.[42,46,76] After eight weeks, the IPCs continue to develop increasing basal and stimulated C-peptide expression over time. It is unclear at what point the endocrine function ceases to increase.

A bioartificial pancreas device must secrete physiologic levels of insulin and respond to glucose stimulation appropriately.[77] To evaluate the glucose-insulin kinetics between BAP devices, we compared glucose and C-peptide stimulation curves (**Figure 9**). The DRC planar macrosheet without a preformed pouch is the only device that demonstrated appropriate glucose-insulin kinetics.[52] The glucose stimulation curve matched the mouse control and the C-peptide levels correlated with C-peptide levels from people with type 1 diabetics who were cured by CIT. The data demonstrates it is possible to develop a BAP with near normal insulin-glucose kinetics in a mouse model. Interestingly, when the DRC BAP was implanted in a preformed pouch, the device had increased glucose-insulin kinetics; the peak blood glucose level was not significantly different from other DRC studies, but the time to return to normal blood glucose levels was faster than the control mouse. Additionally, C-peptide levels were significantly higher than the other DRC devices and the CIT cohort (**Figure 9B**).

In contrast, no device implanted in large animal models achieved a normal glucose response curve compared with the normal pig control[8,53,64,72,74] (**Figure 9A**). The most significant derangement to the glucose response curve is delayed return to euglycemia from peak glucose levels. One potential explanation is that bioartificial pancreas devices implanted in the subcutaneous space are poorly vascularized and reuptake of insulin into the blood stream is delayed. The DRC study using planar macrosheets implanted in preformed vascularized pouches supports this conclusion, as this device demonstrated faster glucose-insulin kinetics than the control. There is a paucity of large animal C-peptide response curve data, but the

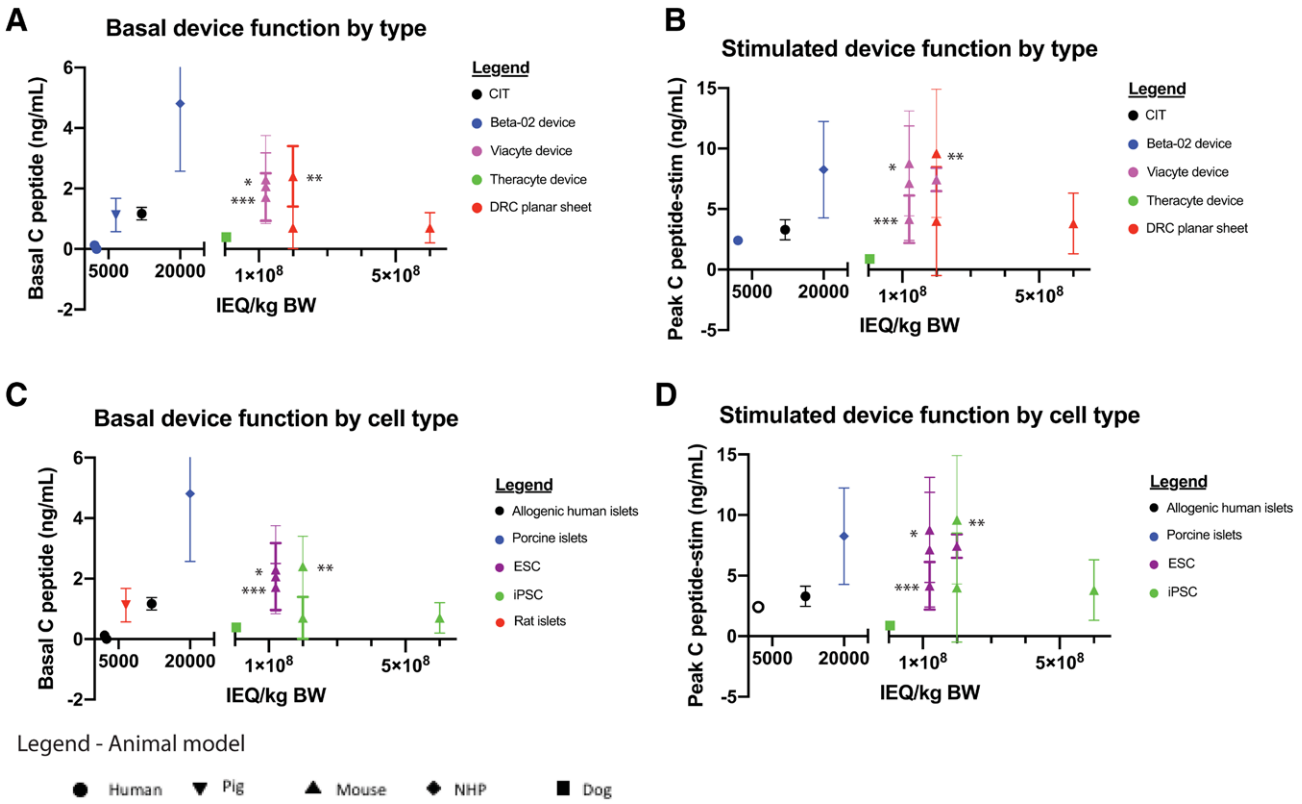

Figure 7. Comparative function of multiple bioartificial pancreas devices in preclinical or clinical trials. A physiologic comparator is indicated by clinical islet transplantation (CIT, black circle, a. and b.) and allogenic human islets (black circle, b. and c.). (A) Basal C-peptide level (ng/mL) *versus* IEQ/kg body weight by device type. (B) Peak stimulated C-peptide level (ng/mL) *versus* IEQ/kg body weight by device type. (C) Basal C-peptide level (ng/mL) *versus* IEQ/kg body weight by indwelling cell type. (D) Peak stimulated C-peptide level (ng/mL) *versus* IEQ/kg body weight by indwelling cell type. The point denoted by *signifies the PEC-01 cell line, **signifies the iPSC-derived IPCs implanted in the preformed pouch, and ***signifies the ESC-derived RA cell line. full color online

C-peptide response curve obtained for the nonhuman primate (NHP) model with the beta-air device is abnormal (**Figure 9B**). Beta air does report stimulated C-peptide levels from one human patient, but these levels are subtherapeutic and did not result in control of circulating blood glucose.

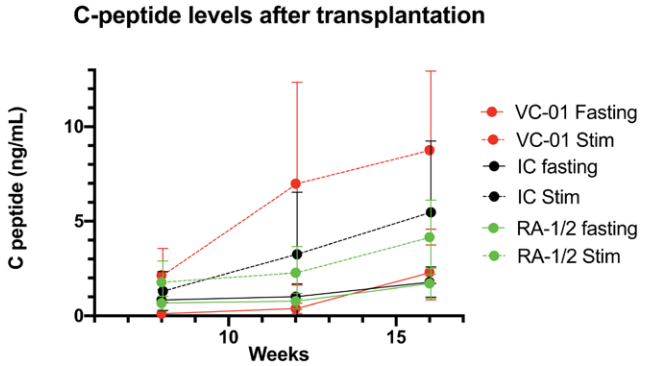

Figure 8. Time-dependent C-peptide release in stem cell-derived islets: Average C-peptide levels produced by implanted stem cell–derived islet-like cells over time, demonstrating a significant average concentration of C-peptide level starting at eight weeks postimplantation, and increasing with time up to the maximum study duration of 16 weeks. VC-01 represents the PEC-01 cell line, having gone through four differentiation stages. The IC cell line is the PEC-01 cell line brought through differentiation stages 5–7. The RA cell line is the IC cell line depleted of pancreatic progenitors. full color online

From the analysis of the most recent BAP studies, we identified areas that require more research to understand their impact on developing a functional and durable BAP. The optimal IEQ/kg BW needs to be established for a BAP device. As ESC and iPSC-derived IPC's become increasingly attractive as renewable islet sources, we need to establish the tumorigenic potential of stem cell–derived IPCs and how to regulate the maturation of an implanted stem cell BAP to achieve an optimal insulin-producing cell mass. Additionally, while subtherapeutic levels of C-peptide are clearly undesirable, more work is needed to understand the potential deleterious effects of supratherapeutic levels of C-peptide. Variables effecting the glucose-insulin kinetics of devices, specifically the downward slope of the glucose response curve, need to be determined.

Conclusion

The ideal bioartificial pancreas is easily implanted, retrieved, protects islets without the need for immunosuppression, and provides long-term pancreatic function. A variety of techniques have been used to encapsulate islets and implantation has been tested in multiple locations. Consistent limitations with all devices are poor long-term islet viability and functionality. The reasons for device failure include hypoxemia, failures in diffusion, inflammatory infiltration of devices, and deranged cell signaling. Although multiple groups are addressing the challenges with oxygenation and proximity to the bloodstream, more work is needed to understand

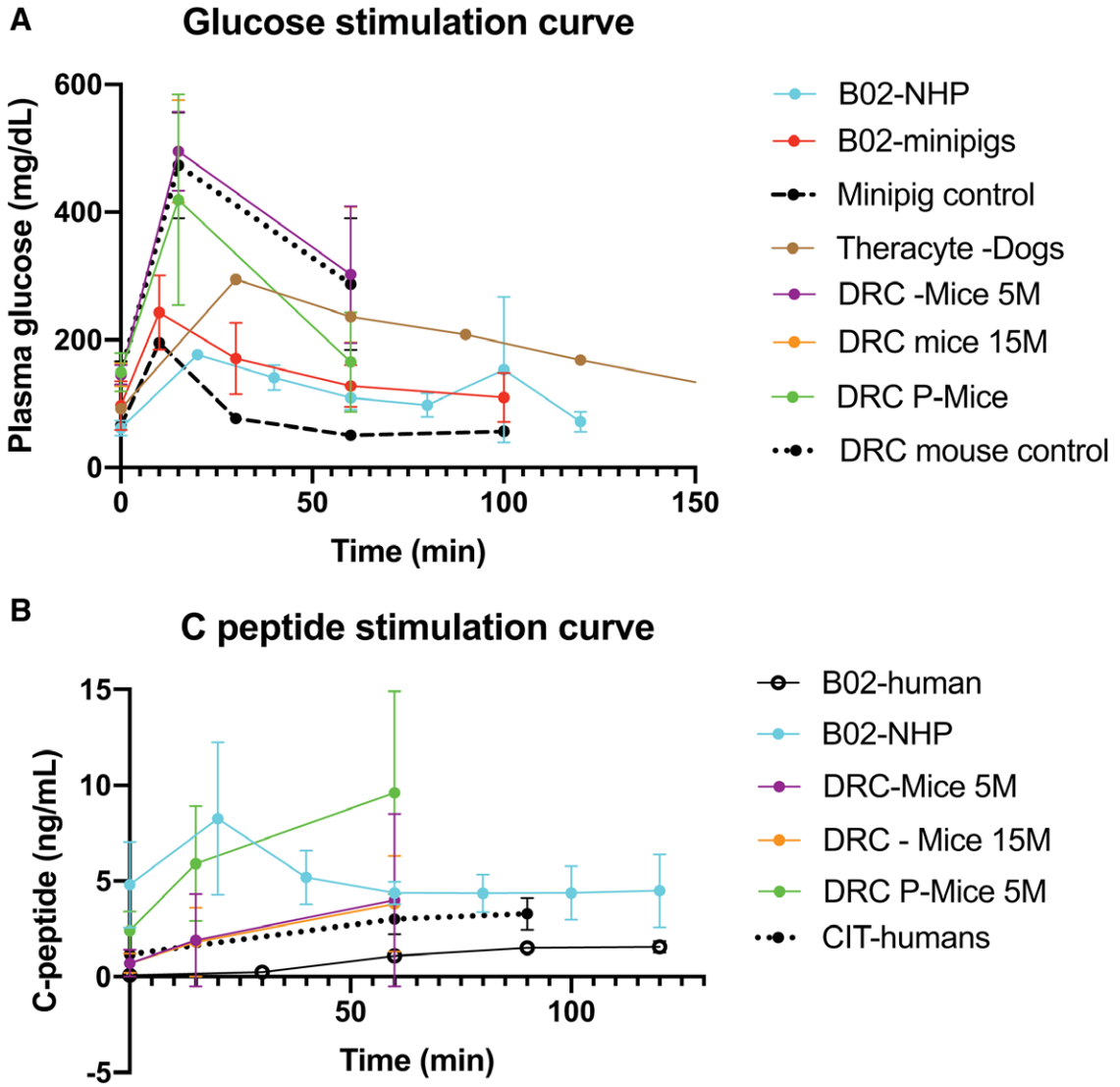

Figure 9. Device function in response to glucose stimulation. DRC 5M represents the Nestle macrosheet device with 1.2×10^8 IEQ/kg body weight. DRC 15M represents the Nestle macrosheet device with 6×10^8 IEQ/kg body weight. DRC P represents the Nestle macrosheet implanted in a preformed pouch. (A) Plasma glucose levels (mg/dL) over 120 min after glucose bolus (B) corresponding plasma C-peptide levels (ng/mL). full color online

other determinants of islet functionality such as deranged cell signaling and accumulation of toxic substances on long-term islet functionality. Recapitulating the native milieu of a pancreas using ECM components will potentially maintain islet functionality and guide stem cell differentiation within scaffolds. Finally, as devices enter human clinical trials, the process of acute and autoimmune rejection of implanted islets must be fully understood and safety must be demonstrated before the bioartificial pancreas can be a feasible cure for a patient with type 1 diabetes.

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