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From islet of Langerhans transplantation to the bioartificial pancreas

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ABSTRACT

Type 1 diabetes is a disease resulting from autoimmune destruction of the insulin-producing beta cells in the pancreas. When type 1 diabetes develops into severe secondary complications, in particular end-stage nephropathy, or life-threatening severe hypoglycemia, the best therapeutic approach is pancreas transplantation, or more recently transplantation of the pancreatic islets of Langerhans. Islet transplantation is a cell therapy procedure, that is minimally invasive and has a low morbidity, but does not display the same rate of functional success as the more invasive pancreas transplantation because of suboptimal engraftment and survival. Another issue is that pancreas or islet transplantation (collectively known as beta cell replacement therapy) is limited by the shortage of organ donors and by the need for lifelong immunosuppression to prevent immune rejection and recurrence of autoimmunity.

A bioartificial pancreas is a construct made of functional, insulin-producing tissue, embedded in an anti-inflammatory, immunomodulatory microenvironment and encapsulated in a perm-selective membrane allowing glucose sensing and insulin release, but isolating from attacks by cells of the immune system. A successful bioartificial pancreas would address the issues of engraftment, survival and rejection. Inclusion of unlimited sources of insulin-producing cells, such as xenogeneic porcine islets or stem cell-derived beta cells would further solve the problem of organ shortage.

This article reviews the current status of clinical islet transplantation, the strategies aiming at developing a bioartificial pancreas, the clinical trials conducted in the field and the perspectives for further progress.

1. Introduction

Type 1 diabetes results from a failure of insulin production by the auto-immune destruction of pancreatic beta cells. While exogenous insulin administration is the cornerstone of the treatment of type 1 diabetes, it is not always able to maintain a satisfactory glycemic control, which contributes to the progressive development of the sometimes devastating secondary complications related to diabetic micro- and macro-angiopathy, and may lead to blindness, lower limb amputation, stroke, myocardial infarction and end-stage renal failure [1]. Maintaining blood sugar levels in a range as normal as possible, thanks to intensive insulin therapy, slows down or even prevents the occurrence of these complications, but at the cost of a higher risk of severe hypoglycemic events [2]. In spite of the advances made in the development of insulin pumps, glucose sensors, and more recently close-loop insulin delivery systems, diabetic complications and severe hypoglycemia still have a significant impact on duration and quality of life in a subset of patients with type 1 diabetes [3]. Such patients are better managed by actually replacing the failing beta cells in order to achieve real-time regulated insulin secretion. This can be achieved very efficiently by transplantation of the whole, vascularized pancreas [4]. The main conclusions of a recent consensus conference were that pancreas transplantation, either performed simultaneously with a kidney or alone, improves long-term patient survival and quality of life of recipients, and may also control the

Abbreviations: αGal, galactosyl-α1,3-galactose; AID, automated insulin delivery; CIT, Clinical Islet Transplant consortium; CITR, Collaborative Islet Transplant Registry; ECM, extracellular matrix; hESC, human embryonic stem cell; hPSC, human pluripotent stem cell; IAK, Islet-after-Kidney transplantation; IBMIR, instant blood-mediated inflammatory reaction; iPSC, induced pluripotent stem cell; ITA, Islet Transplant Alone; NPCC, neonatal porcine pancreatic cell clusters; PERV, porcine endogenous retrovirus; PTFE, poly-tetra-fluoro-ethylene; SHE, severe hypoglycemic events; SIK, Simultaneous Islet-Kidney transplantation

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course of chronic complications of diabetes [4]. Unfortunately, pancreas transplantation is a demanding surgery, associated with a significant morbidity rate [5,6]. This has provided a rationale for developing the concept of islet of Langerhans transplantation as a minimally invasive procedure devoid of the high surgical risks of pancreas transplantation [7]. Thus, two comparable, but not equivalent procedures currently exist for the purpose of beta cell replacement in selected patients with type 1 diabetes.

2. Indications for beta cell replacement

There are certain limitations that prevent to offer pancreas or islet of Langerhans transplantation to each and every type 1 diabetes patient. First, the number of type 1 diabetic patients exceeds by far the number of organ donors. To illustrate this, the incidence of type 1 diabetes in Europe has been estimated at around 150 new cases per year per million population [8]. In comparison, European Union Countries had a rate of 4.2 to 38 organ donors per million population in 2020 [9], or 4 to 36 times less. Second, transplantation of allogeneic organs or cells implies the administration of lifelong immunosuppression to prevent or control the occurrence of rejection. In the case of type 1 diabetes, a disease of autoimmune nature, immunosuppression is also required to prevent recurrence of the disease after transplantation [10]. Therefore, when a beta cell replacement procedure is considered, the benefits must be carefully weighed against the infectious, oncological and other side effects associated with chronic immunosuppression [7,11].

There are currently 2 main situations in which pancreas or islet transplantation is performed. Pancreas transplantation is mostly (75-80%) performed simultaneously with a kidney transplant in patients who have developed end-stage renal failure secondary to diabetic nephropathy [12]. In these patients, immunosuppression is required for controlling rejection of the kidney graft and does not represent a criterion in the risk-benefit assessment. Patients who have a live kidney donor can be transplanted later with a pancreas from a deceased donor and represent the next most common category of pancreas transplant recipients [13]. Simultaneous islet-kidney (SIK) and islet-after-kidney (IAK) transplantation were also by far the most common islet transplant procedures performed until the turn of the millennium [14]. Although less performed nowadays, SIK remains a valuable option for patients with end-stage renal failure and a contraindication to whole pancreas transplantation [7,15]

The second category of patients are those suffering of what has long been referred to as “brittle diabetes” [16], and more specifically those with “problematic hypoglycemia”, i.e. suffering from severe, life-threatening, hypoglycemia episodes, mostly in a context of hypoglycemia unawareness [3,7]. These patients seldom have significant kidney disease and are therefore candidates for an isolated beta cell replacement procedure, since the benefits far outweigh the risks of immunosuppression. Since the publication of the “Edmonton protocol” in 2000 [17], which focused exclusively on patients with problematic hypoglycemia, islet transplant alone (ITA) has become the most frequent islet transplant procedure performed [18]. This is in sharp contrast with whole organ transplantation, pancreas transplant alone consistently representing <10% of all pancreas transplants [12].

3. The islet isolation and transplantation procedure

Before proceeding to transplantation, islets of Langerhans must be extracted from the pancreas procured from a deceased donor by the highly technical method called islet isolation. The method currently used by all islet-producing facilities worldwide is derived with little modification from the automated method developed and described by Ricordi in 1988 [19].

Briefly, the method dissociates the pancreatic tissue into elements of infra-millimetric sizes by the combination of enzymatic digestion and mechanical disruption. The pancreas is injected through the Wirsung duct with a collagenase solution, and placed, together with metallic or ceramic beads, inside a digestion chamber that can be shaken to add a mechanical component to enzymatic tissue dissociation. It is connected to a circulation system and contains a filtration mesh that allows fragments and islets freed from the pancreas to be rapidly removed from the chamber to avoid overdigestion [20]. At the end of the digestion process, the tissue is harvested and purified by centrifugation on a cell separator in order to separate the endocrine tissue from the unwanted exocrine and connective components of the digested pancreas, taking advantage of their lower density compared to exocrine tissue [21].

At the end of the procedure, the islet preparation typically consists in <10 ml of tissue, with 30−90% purity (proportion of endocrine tissue in the final preparation). Considering that islets represent approximately 1% of the total pancreatic volume, on average, a 50-fold enrichment can be obtained. The islet isolation and purification procedure is illustrated in Fig. 1.

For transplantation, the islets, conditioned into a bag, are infused into the portal vein in order to allow them to engraft in the liver. The portal vein is usually catheterized by interventional radiology, using a percutaneous, trans-hepatic approach [22].

Intrahepatic islet transplantation has been associated with an approximate rate of 10% significant procedure-related morbidity [23,24]. The most frequent reported adverse event is a transient elevation of liver function tests, but is usually devoid of clinical significance [25,26]. Of the potentially severe complications, the most common is bleeding from the trans-hepatic tract, causing hemoperitoneum or intra-hepatic hematoma. Portal vein thrombosis is the most feared, but thankfully rare, complication [27].

Portal vein access by laparotomy, with catheterization of a mesenteric venous tributary, has been described and represents an alternative to the percutaneous approach [28,29]. This method can be very useful for patients presenting high risk of bleeding, thrombophilic disorders or if interventional radiology is not available. The open surgical approach has demonstrated similar results as percutaneous access in terms of function, and similar or even lower complication rates [28,29]. The more invasive nature of the surgical approach can however be a drawback.

4. Outcomes of clinical islet transplantation

Thanks to spectacular improvements in functional outcomes, islet transplantation has evolved to become a clinical reality, and is part of the standard of care for patients with complicated type 1 diabetes. Outcome data can be obtained from 3 types of sources: registries, multicenter clinical trials, and single center retrospective reports. The collaborative islet transplant registry (CITR) has the advantages and drawbacks of all registries, namely large numbers of subjects and a reflection of the “real life” situation, but low data granularity [18]. The CITR collects data from all centers in America and Australia, but only a few selected centers in Europe, introducing a bias that makes it problematic to extrapolate to the true global picture. In particular, data on the outcomes of SIK transplantation are conspicuously absent. In the CITR, the overall 5-year insulin independence rate is 25.2% and 21.0% for ITA and IAK respectively. This outcome figures are lower than those of whole pancreas transplantation, but this should be put in perspective with the specific profiles of candidates for islet transplantation, i.e. patients with problematic hypoglycemia and/or contraindications to pancreas transplantation [7]. Islet transplantation is exceedingly efficient at abolishing the occurrence of severe hypoglycemic events (>90% at 5 years) and controlling HbA1c (Fig. 2).

Three multicenter prospective clinical trials have been conducted in recent years. The US Clinical Islet Transplantation (CIT) consortium has published the results of 2 trials evaluating the efficacy of islet

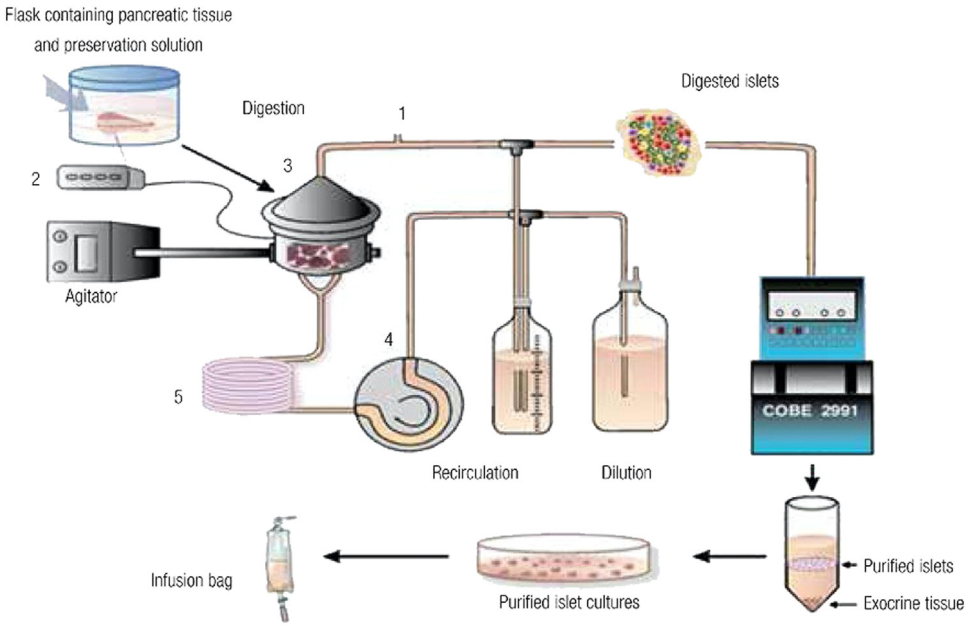

Fig. 1. Schematic representation of the automated method for islet isolation and purification. 1: sampling location for islet collection and for the monitoring of digested islets; 2: chamber temperature monitoring probe (37 °C); 3: Ricordi chamber with beads, pancreatic tissue and collagenase solution; 4: peristaltic pump to ensure the circulation of the solutions in the system; 5: heating coil in a hot water bath. Reproduced from: [20], under CC BY-NC 3.0 license.

transplantation in ITA and IAK cohorts [30,31]. They were phase 3, prospective, open-label, single-arm studies, assessing the impact of islet transplantation alone in patients with hypoglycemia unawareness, or previously transplanted with a kidney for end-stage diabetic nephropathy and with poor metabolic control. The composite primary endpoint, eradication of severe hypoglycemic events (SHE) and HbA1c<7% at 1 year after the first islet transplant, was identical in both studies. Remarkably, the primary endpoint was achieved in 87.5% of ITA subjects and 62.5% for IAK, and was maintained in 71% and 58% at 2 years, respectively. Of the secondary endpoints, insulin independence was achieved in 52% and 37.5% at 1 year, and eradication of SHE at 2 years was achieved in 93% and 75% [30,31].

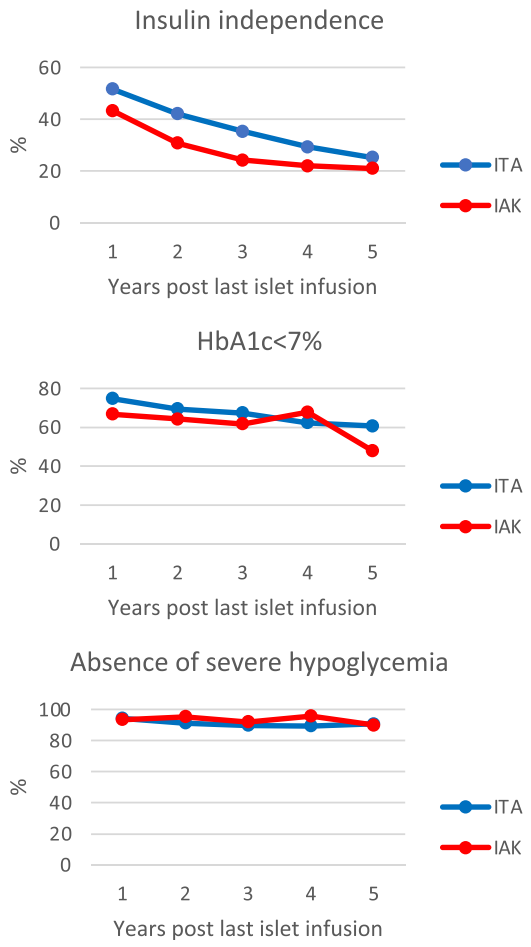

Fig. 2. Outcomes of islet transplantation reported to the Collaborative Islet Transplant Registry. Adapted from [18].

The TRIMECO trial was the first randomized trial comparing transplantation to a reference treatment for any type of organ failure. The TRIMECO trial enrolled 50 patients in 15 institutions in France between 2010 and 2013. Study subjects were either patients with hypoglycemia unawareness (ITA) or patients previously transplanted with a kidney for end-stage diabetic nephropathy with poor metabolic control (IAK). This was a phase 3, open label, two-arm, prospective randomized trial. Subjects were randomized 1:1 to receive immediate islet transplantation (experimental arm) or intensive insulin therapy (control arm). This trial was conducted with a network approach, in which 3 centralized facilities in France and Switzerland isolated islets for the 15 participating centers. At 6 months, mean HbA1c was 5.6% vs 8.2%, fasting blood glucose was 5.2 mmol/l vs 5.7 mmol/l; 84% vs 0% had HbA1c<7% and abolition of SHE. At 1 year, 59% were off insulin, median beta score was 7, median HbA1c was 5.8%, and 70% of patients had a HbA1c<7% and absence of SHE [32]. This confirmed the excellent results of islet transplantation and showed that the effects of intensive insulin therapy were present but unable to match those of islet transplantation.

There are only two published reports of 10-year outcomes outside of the CITR. The Lille group reported on a prospective parallel-arm cohort study of 28 patients transplanted with ITA or IAK. The primary outcome (insulin independence with A1C ≤6.5%) was met in 39% and 28% of subjects at 5 and 10 years. Graft function, evidenced by persistence of circulating C-peptide, was achieved in 80% in the long term, and associated with improved glycemic control and marked decrease of SHE [33]. The GRAGIL network reported the only actual 10-year results available, albeit in a retrospective study. Ten years after islet transplantation, median HbA1c was 7.2% versus 8.0% before

transplantation. Seventeen of 23 (73.9%) recipients were free of severe hypoglycemia. Insulin requirements (UI/kg/day) were 0.3 and 51.9% recipients had a functional islet graft at 10 years. With a 10-year follow-up in a multicentric network, islet transplantation provided sustained improvement of glycemic control and was efficient to prevent severe hypoglycemia in almost 75% of recipients [34].

5. Challenges of allogeneic islet transplantation

Although the reported outcomes of islet of Langerhans transplantation show a clear improvement over standard insulin therapy, they are far from meeting those of whole pancreas transplantation, especially in the long term [12]. The two major issues met by islets are poor engraftment and progressive attrition of functional mass. Several causal factors have been identified and basically occur during the islet isolation procedure, at the time of engraftment or at the implantation site [35]. The most conspicuous consequence is the need for multiple donors, typically 2–3, to achieve the outcomes described above [18].

First, the isolation procedure implies the separation of the endocrine from the exocrine pancreas by enzymatic digestion. In addition to the direct cell loss induced by this aggressive process, the procedure disconnects the islets from their vasculature and innervation, disrupts their extracellular matrix (ECM), leading to ischemic damage and anoikis-mediated apoptosis [36,37].

Second, engraftment in the liver is impaired not only by the absence of immediate vascularization, but also by the pro-inflammatory nature of the micro-environment in which islets are implanted [38]. In particular, islet transplantation into the portal venous system induces an “instant blood-mediated inflammatory reaction” (IBMIR). This inflammatory process is triggered by the intravascular contact of islet cells and tissue factor and involves activation of the complement and coagulation cascades, ultimately resulting in clot formation and infiltration of leukocytes into the islets, leading to islet destruction [39]. IBMIR is thought to contribute to the immediate loss of anything between 20 and 80% of the implanting islets and is considered the root cause of poor islet engraftment [35]. The consequence is that, even after transplantation of islets obtained from multiple donors, only a marginal functional endocrine mass has been able to implant.

The third challenges faced by transplanted islets continuously occur over time, leading to progressive functional attrition. Because of the marginal implanted mass, the metabolic workload on each individual islet is significant and sustained and may lead to progressive graft exhaustion [35,40]. Implanted islets are also subject to local glucotoxicity, lipotoxicity and toxicity of immunosuppressive drugs. Finally, immune rejection and recurrence of autoimmunity also threaten islet graft survival [35].

Some of these challenges have been successfully addressed in the past decades, which has led to a steady improvement of clinical islet transplantation outcomes [41]. However, many of these challenges are there to stay and face the field of islet transplantation, unless a significant paradigm shift is made in the approaches to beta-cell replacement.

6. The artificial pancreas

The artificial pancreas could be a fully technological solution to these issues. Schematically, the components of an artificial pancreas are a glucose sensor, an insulin-infusing pump and a device able to receive signals from the sensor, compute the required insulin dose to administer thanks to an algorithm and send signals to the pump for insulin delivery [42]. A fully efficient artificial pancreas, or more correctly automated insulin delivery (AID) system, must continuously measure glucose levels and be able to calculate and continuously administer proper insulin doses in order to adjust glycemia to an ideal range in real-time. Ideally, the whole system should be able to function in a closed loop and autonomously, i.e. without external intervention from the patient or a physician.

This is a field that has seen rapid advances in the past decade, and various models of AID systems, combining different insulin pumps, glucose sensors and algorithms, have been authorized and are available on the market in several countries benefiting from health care coverage. Commercially available devices are in fact “hybrid closed loop systems”, since they allow, or rather require, patients equipped with the system to interact with the system, in order to adjust insulin dose delivery at the times of meals or exercise.

AIDs have been tested in randomized controlled trials, mostly in adults and adolescents, but in some cases also in pediatric patients [43,44]. Overall, times in range (3.9–10 mmol/l) of 65–75% have been reported from RCTs, with times in hypoglycemia (<3.0 mmol/l) of 0.2–0.8% [45]. These results are of course remarkable but were largely obtained in subjects in which severe hypoglycemia was not an inclusion criterion, and in some cases was even an exclusion criterion [43,46,47]. As a benchmark, islet transplantation was able to achieve time in range in 94% with a 3-year follow-up and times in hypoglycemia of 0% [48].

Although current hybrid closed-loop systems have been shown to improve time spent in the target glycemic range compared to standard therapy, they are still not reactive enough to efficiently and reliably control glycemia without user input regarding meal timing, carbohydrate intake and time and extent of exercise. Efforts are ongoing to develop new “intelligent” algorithms that will allow to solve these issues and “close the loop”. Although AIDs have the major advantage to function without requiring chronic immunosuppression, they are far from achieving the same outcomes as islet of Langerhans transplantation. They also have acceptance and usability issues from patients, including acceptance of wearing an external device, range values that may be considered as suboptimal and workload for the estimation of the proper actions to take at times of meals or exercise [49,50].

There are several reasons that may prevent AIDs to ever reach the level of glycemic control achieved by an islet graft, let alone fully functional native islets. One consideration pertains to delays both in the measurement of glucose levels and in insulin delivery. Sensors measure glucose in the interstitial fluid, in which there is a time lag before levels equalize with those in the blood [51]. Similarly, there is a delay before subcutaneously injected insulin reaches the circulation [52]. The response delays, in detection and injection, both inherent to the subcutaneous route are likely to remain a challenge for the development of more powerful algorithms [53].

Another consideration is that, more than an “artificial pancreas”, AIDs are in effect an “artificial beta cell”, detecting glucose and releasing insulin, whereas beta cells exist within the complex structure of an islet of Langerhans, themselves distributed inside a well-organized organ, in which cross-talk between different types of cells determines their function, survival and proliferation [54].

Research efforts in the development of bi-hormonal systems, delivering both insulin and glucagon in real time, are ongoing, but a true advantage over AIDs is as yet unclear [55,56]. Other efforts involve the integration of islet-based biosensors in the device but have not yet reached the clinical trial stage [57].

Overall, the impossibility of reproducing all the components, interconnections and signaling seen in these two structures, the islets and the pancreas, may in fact represent an insuperable obstacle for the AID field in its quest to achieve perfect glucose control.

7. The bioartificial pancreas: what it is and how to build it

The only strategy able to achieve the goal of perfect glycemic control in type 1 diabetes, while resolving the issues of organ (or cell/tissue) shortage and need for lifelong immunosuppression resides in the development of a bioartificial pancreas. The term “bioartificial

pancreas" was first coined in 1980 to describe the technical solution of encapsulating native islets of Langerhans in a semipermeable, immunoprotective and biocompatible membrane [58]. For many years, and encouraged by a seminal report of insulin independence after transplantation of encapsulated islets in a diabetic patient [59], a result that has never been reproduced since, attempts at developing a bioartificial pancreas have almost exclusively focused on the micro-encapsulation of native islets inside alginate beads [60,61]. Unfortunately, promising results achieved in rodent or even larger mammal models have failed to translate into clinical success [62]. Two major factors for these relative failures have been identified, namely poor biocompatibility,leading to an inflammatory reaction around the implanted capsules, and hypoxia secondary to the thickness of the capsule and inflammatory tissue surrounding the islets, which acts as a barrier to oxygen and nutrients [62].

While this experience has been of great value to investigators in the field, it has become obvious that, to be effective, a functional bioartificial pancreas will require a much more sophisticated design that should respond to the following requirements: (i) long-term (if possible lifelong) physiologic insulin delivery, (ii) mechanical protection, (iii) biocompatibility, i.e. no or minimal inflammatory response, (iv) rapid revascularization to ensure adequate oxygenation, (v) adequate microenvironment, (vi) immune protection, (vii) easy implantation, accessibility/retrievability, and (viii) applicability to infinite sources of insulin-producing tissues [37,61]. In particular, some of the interest has shifted from the concept of immune protection by an immune-isolating physical barrier to incorporation of the insulin-secreting cells into an immunomodulatory biomaterial [63].

Constructing a bioartificial pancreas meeting this extensive set of criteria therefore requires to address 4 main issues: 1) what types of cells of tissues will be utilized as a source of insulin, 2) what encapsulation strategy will be utilized, 3) what types of (bio)materials or accessory cells will be utilized to provide the adequate microenvironment, and 4) what will be the optimal site to implant the construct?

The overall concept for a bioartificial pancreas is schematically represented in Fig. 3.

8. The bioartificial pancreas: encapsulation strategies and materials

Strategies for islet encapsulation can be separated in 3 types: macro-, micro- and nano-encapsulation [61]. Macro-encapsulation refers to the integration of several islets or islet-size tissues into a construct that can be as much as a few centimeters in dimension, this strategy has the advantage of versatility in terms of shape, and type and structure of the matrix or scaffold materials that can be integrated, to mimic the islet extracellular matrix, for example. It also offers the possibility to add to the construct other cell types that may help reproduce a natural microenvironment or provide their immunomodulatory properties. Its drawbacks are obviously the need for vascular connections to ensure proper levels of oxygenation [64]. Some techniques at providing a fully biologic vascularized "container" for the islets have been explored, with some success, in small animal models. These include isolated decellularized small bowel segments or "venous sacs" (isolated segments of vein vascularized by the vasa vasorum) [65,66]. Although only mildly related to macro-encapsulation, these strategies have improved islet re-vascularization, but obviously without providing immune-protection.

Micro-encapsulation, overwhelmingly utilizing alginate as the encapsulation material, has been the strategy of choice over several decades, albeit with very limited success other than in rodent models [67–70]. As mentioned above, this has largely been the result of sub-optimal biocompatibility and oxygen permeability of the alginate capsule. Inadequate microenvironment may also be a key issue, that could be resolved by engineering the alginate into a hydrogel containing the key components of the islet extracellular matrix [71]. Additionally, retrievability of micro-encapsulated islets has been extremely difficult [70]. Nano-encapsulation, or conformal coating has been more recently proposed as a method for conferring immunological protection to the islet, while allowing easy oxygen and nutrient inflow and insulin outflow, thanks to an ultra-thin layer of encapsulating material [61]. The whole point of nano-encapsulation is obviously not to confer mechanical protection, but rather to provide immune isolation or immune camouflage thanks to the coating of the islets with a biofilm able to release immunosuppressive pharmacological agents, allow cytokine capture or enable delivery of immunomodulatory signals [72,73].

Materials utilized for islet encapsulation have largely consisted in hydrogels of natural polymers, with several configurations of alginate as the material of choice. Polymer fabrication determines a pore size that should be large enough to allow oxygen in and insulin out, but small enough to prevent contact with immune cells, but also toxic reactive oxygen species or pro-inflammatory cytokines, which is difficult to achieve given the similar sizes of insulin and pro-inflammatory compounds [63]. At least in part for these reasons, clinical attempts at transplanting alginate-microencapsulated islets have led to disappointing results [68–70].

Beside alginate, a wide range of encapsulating materials have been suggested or utilized, namely other natural polymers (collagen, agarose), synthetic polymers (polyethylene glycol, polyelectrolytes,...) or inorganic materials (silicon, aluminum oxide,...) [61,73].

9. The bioartificial pancreas: sources of insulin-producing tissue

An ideal bioartificial pancreas should at the same time confer immune protection to the functional cells and utilize an unlimited source of insulin-producing tissue. This double constraint has led research groups to focus either on establishing cell sources meeting the necessary safety and efficacy requirements, or on optimizing bioengineering strategies. For this reason, the early steps taken by most tissue engineering groups has been to use native islets. This has been the case for early attempts at clinical transplantation of microencapsulated islets [68–70], and for subsequent clinical trials testing biologic scaffolds [74] or macro-devices [75–77].

It should be noted that islets of Langerhans exhibit a large variability in size, ranging from <50 um to 500 um or more. The islet isolation procedure disconnects the islets from their microenvironment, their extracellular matrix, but also interrupts their vascularization [37,78,79]. Among other hurdles, islets of Langerhans must rely on passive diffusion to ensure their oxygenation after transplantation, leading to a necrotic core that can be observed in the larger islets [78,80]. Indeed, smaller size islets have been shown to display superior function in comparison to larger ones, despite their higher beta cell mass [81]. For this reason, efforts have been made in generating islet spheroids -or "pseudo-islets"- of uniformly small size by re-aggregation of the endocrine cells after enzymatic dissociation of the islets [82,83].

There have been interesting attempts at co-transplantation of islets or islet cells with other cell types, most recently parathyroid or amniotic epithelial cells [84,85]. The intended role of these supporting cells is to re-establish certain signaling pathways promoting function, survival or revascularization. A large number of cell types have shown beneficial impacts on islets in co-culture experiments [86]. These observations have led to the integration of other cell types into single insulin-producing units, or islet cell organoids [87]. Islet cell organoids are hybrid spheroids constructed from native islet cells, or insulin-producing cells of other sources, re-aggregated with other cell types (accessory cells) that would offer support, restore extracellular matrix, reestablish signaling pathways, enhance insulin response or confer immune modulation or cytoprotection [37,82,86,88].

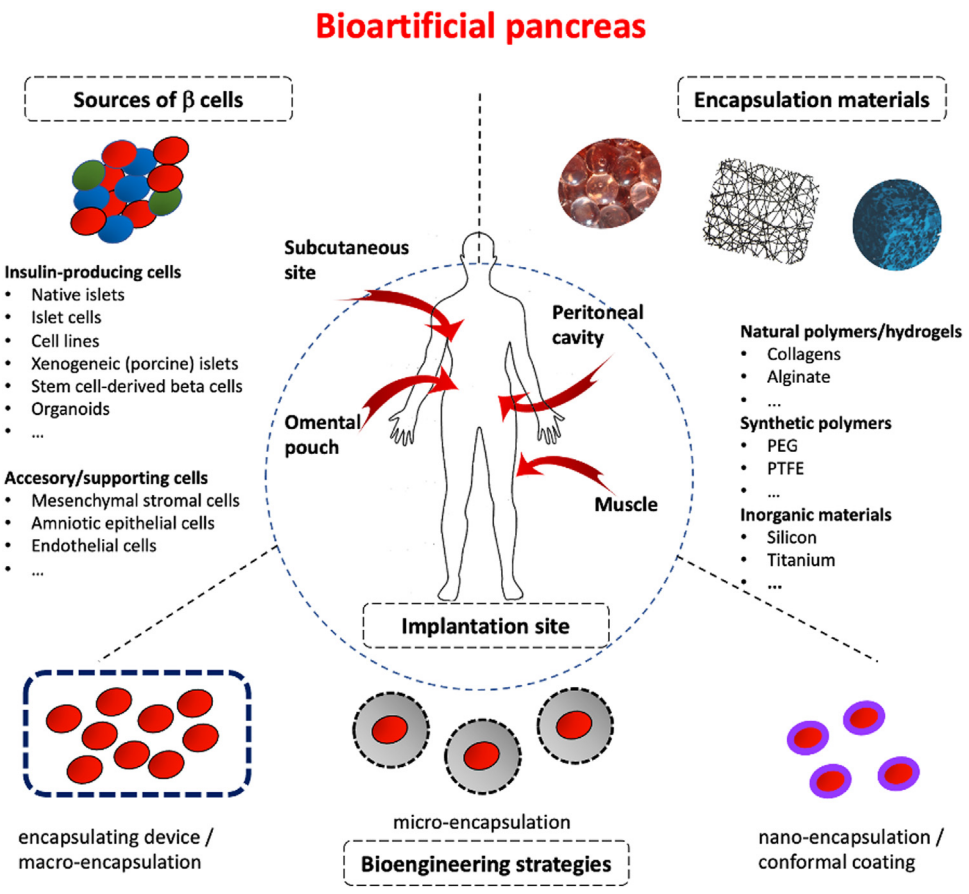

Fig. 3. General concept for a bioartificial pancreas.

As already mentioned above, the major issue of islets of Langerhans or islet cells is their limited availability. The most likely sources for large scale availability of insulin-producing tissue are xenogeneic islets of porcine origin and stem cell-derived beta cells.

Historically, before the availability of synthetic insulins, patients were treated for decades with porcine insulin, which differs by only 1 amino acid from the sequence of human insulin [89]. This remarkable similarity and the relative ease in breeding large litters have built up the rationale for using pigs as an "unlimited" source of xenogeneic islets. Additionally, since porcine islets obviously do not express human MHC antigens, they also have the potential to avoid allosensitization and recurrence of MHC-restricted recurrence of autoimmunity. Various sources of porcine islets have been explored, from fetal porcine islet cell clusters, to neonatal porcine pancreatic cell clusters (NPCC), to adult mature porcine islets [90–92].

The two main hurdles for the successful implementation of clinical islet xenotransplantation are the potential for transmission of porcine endogenous retroviruses (PERVs), but mostly the immunologic barrier, which may lead to hyperacute rejection or inflammatory damage, in particular IBMIR, as well as chronic rejection [93]. Long-term insulin independence in pig-to-non-human primate transplantation has been reported by the groups at the Universities of Minnesota and Emory [94,95], and more recently at Seoul National University in Korea [96–98]. These results were obtained with more or less conventional immunosuppression, using islets from unmanipulated pigs [94–98]. The generation of pigs genetically modified to circumvent the major mechanisms of immune and inflammatory damage to xenogeneic organs has been a major field of research and development, one of the first targeted molecules being the galactosyl-α1,3-galactose (αGal) epitope, against which humans naturally produce antibodies responsible for hyperacute rejection [99]. Since then, the complexity of the mechanisms involved in xenogeneic destruction has led to the generation of multi-transgenic pigs, thanks to the development of gene editing technology, to circumvent the many issues faced in islet or whole organ transplantation [100,101]. The availability of multi-transgenic animals has recently led to the spectacular reports of pig-to-human heart and kidney transplants [102]. The perspectives for xenogeneic islets to be the functional part of a bio-artificial pancreas have therefore never been higher.

Human pluripotent stem cells (hPSCs) are obtained either from the inner cell mass of the blastocyst (human embryonic stem cells (hESCs) or generated from adult somatic cells by forced expression of a specific set of reprogramming factors (induced pluripotent stem cells (iPSCs). hESCs and iPSCs both display pluripotency and differentiation potential and hold immense promise in the field of regenerative medicine and particularly in cell therapy for type 1 diabetes [103,104]. With recent advances in the field, several groups have reported generation of hPSC-derived beta cells with characteristics closely resembling those of *bona fide* beta cells [105,106]. Several protocols mimicking the steps of the in vivo development of the embryonic pancreas have been developed for *in vitro* differentiation of hPSCs either into insulin-secreting beta cells or into their pancreatic progenitors [107–110]. Clinical trials have been initiated with 2 different hESC-derived cell products. ViaCyte, has transplanted, in type 1 diabetic subjects, pancreatic progenitor cells encapsulated in a poly-tetra-fluoro-ethylene (PTFE) device, and demonstrated safety, engraftment and endocrine function [111,112]. More recently, Vertex has initiated a trial in which fully differentiated hESC-derived islet cells are transplanted intraportally. Briefly, patients with unstable type 1 diabetes were transplanted with VX-880, investigational stem cell-derived, fully differentiated pancreatic islet cells, without encapsulation, and therefore with immunosuppression (NCT04786262).

The first 3 transplanted subjects have shown partial or full (insulin-independence) graft function, a marked improvement in glycemic control and no serious adverse events have been observed [113,114]. Vertex is working on the development of an encapsulating device to be utilized in the future as a bioartificial pancreas with the VX-880 cell therapy product.

Pancreatic islets are well vascularized mini-organs assembled as 3D spheroids, with their own innervation and complex intercellular communications [115]. Islets are not only composed of insulin-producing and other endocrine cells, but also a variety of accessory/supporting cells [116]. The complex cytoarchitecture, reciprocal coordination and balancing intercellular interactions within islets are key factors for the maintenance of proper beta cell function [117−120]. Therefore, generation of complete islet-like 3D structures, or islet organoids [87], rather than differentiating single cells into a specific type, appears to be a prerequisite for achieving physiologic glucose homeostasis in a cell therapy strategy for type 1 diabetes [37].

In summary, the functional, insulin-producing, components of a bioartificial pancreas can be native human or xenogeneic islets or islet cells, stem cell-derived cells, spheroids or organoids derived from any of the above.

10. The bioartificial pancreas: sites of implantation

Clinical islet transplantation is performed by intraportal infusion of the islets, which subsequently engraft inside the liver. Although it has been near-impossible to find more favorable alternative sites for islet transplantation, the liver has been considered as suboptimal because of the numerous insults sustained by the islet grafts in a notoriously pro-inflammatory micro-environment [38]. As a consequence a variety of extra-hepatic sites (skeletal muscle, omentum, bone marrow, gastric submucosa,..) have been proposed, and clinically tested with various levels of success, for human islet transplantation [121,122].

Obviously, with the possible exception of a nanoencapsulation strategy, intraportal infusion of a bioartificial pancreas is not feasible because of size considerations, making the identification of a suitable extra-hepatic site a necessity. An ideal implantation site for a bioartificial pancreas should offer easy access, for implantation, monitoring and retrieval, allow easy revascularization and have a minimally pro-inflammatory microenvironment [123]. So far, clinical attempts at transplanting encapsulated islets have been largely restricted to the subcutaneous space, the peritoneal cavity and the omentum [123]. The subcutaneous space is a favored site because of its ease of access and in spite of its lack of vascularity and low oxygen tension [124]. Efforts at overcoming this, using prevascularization strategies or embedding in matrices preventing intra-islet hypoxia, have been successful in rodent and even non-human primate models of islet or hPSC-derived cell transplantation [125−128]. Overall, all research efforts are pointing toward the subcutaneous space as the optimal site for implanting a bioartificial pancreas.

11. The bioartificial pancreas: clinical trials

Historically, the first successful attempt at transplanting a “bioartificial pancreas”, as rudimentary as the strategy was, was the report by Soon-Shiong in 1994 of insulin independence achieved 9 months after intraperitoneal transplantation of alginate-encapsulated islets in a type 1 diabetic patient who was already on immunosuppression for a previous kidney transplant [59]. Further attempts at transplanting human islets obtained from cadaveric organ donors, encapsulated in various alginate formulations, and transplanted without immunosuppression, were not able to reproduce the same success, with at best a slight temporary decrease in insulin requirements and detection of C-peptide in the blood or urine [68-70,129].

The group at the University of Dresden in Germany has transplanted human islets inside an oxygenated subcutaneous device (Beta-O2 device) [130] in a single patient. Briefly the device consists of a container with a PTFE semi-permeable membrane inside which islets are embedded in an alginate hydrogel. The device is refilled daily, through a tubing system with an oxygen-rich gas mixture. Persistent graft function was demonstrated up to 10 months, but at only minute levels of circulating C-peptide and no impact on metabolic control. Safety was demonstrated as well as preservation of islet morphology and function after retrieval of the device [131]. The same device was tested by the University of Uppsala on 4 patients with essentially identical outcomes [77].

A few clinical trials using porcine islets have taken place in the past 2 decades. Valdes-Gonzalez et al. in Mexico transplanted NPCCs [92,132] in a subcutaneous collagen-covered device, implanted 2 months before transplantation in order to allow pre-vascularization of the graft site. NPCCs were co-transplanted with neonatal porcine Sertoli cells [133] for their immunomodulatory properties and no immunosuppression was administered. Study subjects (N = 23) were young adults or adolescents. While no case of insulin-independence was reported, no serious adverse events were reported, evidence of function (positive porcine C-peptide in the urine) was observed in the long-term and a majority of subjects had significant reductions of their insulin requirements [134]. Elliott et al. in New Zealand have reported a single case of intraperitoneal transplantation of alginate-encapsulated fetal porcine islet cells, without immunosuppression, in a patient with type 1 diabetes who demonstrated a mild decrease in daily insulin requirements and HbA1c, and evidence of porcine C-peptide in the urine for almost one year. Nine years later, some encapsulated islets were recovered by laparoscopy and the presence of some live and functional islets could be demonstrated [135]. More recently, Matsumoto et al. have reported the results of 2 trials NCT00940173, NCT01739829) performed in Argentina, in which NPCCs were encapsulated in alginate beads and transplanted in the peritoneal cavity of 8 and 14 type 1 diabetic subjects, without immunosuppression. A lasting impact on HbA1c was shown in the second study with >2 years follow-up [136,137].Overall, although none of the patients transplanted with encapsulated islets have achieved insulin-independence, these trials -remarkably all conducted without immunosuppression- offer favorable perspectives for a xenogeneic bioartificial pancreas, in view of the new availability of multi-gene-edited pigs [102] and the potential for improvement of encapsulation strategies.

The first-in-human phase 1/2 clinical trials of hESC-derived islet cell transplantation were conducted by the ViaCyte company. Pancreatic endodermal cells (PEC-01), encapsulated in expanded polytetrafluoroethylene devices, were used as the insulin-producing source. In the first trial (VC-01; NCT02239354), the PEC-01 cell product was encapsulated in the PTFE Encaptra® device, without immunosuppression. The device was shown to be safe and well tolerated. Interestingly, the presence of insulin-positive cells was demonstrated in retrieved devices up to 24 months after implantation, but only trace amounts of circulating C-peptide could be detected in some of the study subjects, mostly for biocompatibility issues [111] .

In the second trial (VC-02; NCT03162926), portals were drilled in the device to allow vascular ingrowth, which implied contact with the immune system and required immunosuppression of the study subjects. Better differentiation of implanted cells was achieved and better biocompatibility was observed in the VC-02 trial, thanks to the modification of the encapsulating device allowing direct vascularization of the grafts, but again only trace amounts of C-peptide could be measured in about one third of study subjects in the months following implantation. Nonetheless, these first attempts at clinical transition have generated strong preliminary evidence that encapsulated stem cell-derived islet products have the capacity to differentiate, survive and exhibit signs of function in patients with type 1 diabetes [112].

12. The potential of amnion-derived components in building a bioartificial pancreas

Our group at the University of Geneva has long been interested in amniotic-derived products as potential accessories in the construction of a bioartificial pancreas. Amnion-derived products have been used for decades as a biomaterial for wound care or tissue replacement, but also because of their immunomodulatory capacities. Amniotic cells possess the ability to modulate and suppress the innate and adaptive immune systems, making them a potential therapeutic tool in chronic inflammatory disorders and for the induction of tolerance in transplantation models. They also exhibit multilineage differentiation capacity making them a promising cell source for tissue engineering [138]. Additionally, the amniotic membrane is a component able to provide extracellular matrix (ECM) that is near-identical in composition to the islet ECM [37,88,139]. We have generated viable and functional islet organoids from dissociated islet cells and human amniotic epithelial cells (hAECs). The incorporation of hAECs into islet organoids markedly improved in vitro and in vivo function, engraftment, viability and graft function in a murine model of diabetes in comparison to native islets or spheroids made of islet cells alone, including in hypoxic stress conditions and was linked, at least in part to better graft revascularization [140]. The gene expression patterns and pathways involved in the immunomodulation and cytoprotection conferred to islet cells by hAECs were studied in-depth, demonstrating a role for HLA-G, HLA-E and PDL-1 gene expression and the involvement of the JAK1/2 - STAT1/3 and the NF-κB1 pathways [141].

In a next set of experiments, we have demonstrated the additional benefit of pre-vascularizing the islet organoids thanks to the incorporation of endothelial cells into the constructs [142]. Again, beneficial effects were shown both in vitro and in vivo, and we could show that these effects occurred through a cross-talk between hAECs, endothelial cells and islet cells, mediated by the upregulation of genes promoting angiogenesis and β cell function [142]. Finally, we have developed a hydrogel, derived from the amniotic membrane and again demonstrated improvement in vitro and in vivo in the function and survival of human islets embedded in the hydrogel [143].

The ultimate purpose of the Geneva approach is to combine these elements, i.e. embedding islet organoids obtained as described above into the amniotic membrane-derived hydrogel, utilizing them as building blocks for the construction of a bioartificial pancreas [37,139]. The long-term vision is to generate the islet organoids from xenogeneic islet cells or stem cell-derived beta cells, rather than cadaveric human islets.

13. Conclusions

Beta cell replacement as a cure for type 1 diabetes is a rapidly progressing field. Advances are being observed in 3 different fields of research, i.e. encapsulation strategies, porcine xenotransplantation and regenerative medicine. These advances will be critical for the delivery of a long-promised therapeutic solution that would be available to all patients suffering from type 1 diabetes, including children, before they reach the stages of diabetic complications, and without the hurdles of organ shortage and the burden of immunosuppression.

Declaration of Competing Interest

None to declare.

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