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Encapsulated islets for diabetes therapy: History, current progress, and critical issues requiring solution ☆

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ABSTRACT

Insulin therapy became a reality in 1921 dramatically saving lives of people with diabetes, but not protecting them from long-term complications. Clinically successful free islet implants began in 1989 but require life long immunosuppression. Several encapsulated islet approaches have been ongoing for over 30 years without defining a clinically relevant product. Macro-devices encapsulating islet mass in a single device have shown long-term success in large animals but human trials have been limited by critical challenges. Micro-capsules using alginate or similar hydrogels encapsulate individual islets with many hundreds of promising rodent results published, but a low incidence of successful translation to large animal and human results. Reduction of encapsulated islet mass for clinical transplantation is in progress. This review covers the status of both early and current studies including the presentation of corporate efforts involved. It concludes by defining the critical items requiring solution to enable a successful clinical diabetes therapy.

Contents

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1. Introduction

1.1. Historic antecedents to islet encapsulation

1.1.1. Diabetic antiquities

As an introduction to his MD Thesis in 1983 and in a later publication, Richard Downing has presented a well-studied history of diabetes and insulin-producing cell transplantation. It is summarized in part here to explain the important developments that resulted in islet encapsulation [1,2]. The signs of diabetes were described in clinical terms in antiquity starting with polyuria and polydipsia in the Ebers Papyrus dated 1500 B.C. [3] followed by Hippocrates (469–399 B.C.), Aritstotle (384–322 B.C.), Celsus (30 B.C.–50 A.D.), and Galen (129–199 A.D.) [4,5]. Aretaeus the Cappadocian (30–90 A.D.) named the disease "diabetes" from the Greek word for "siphon" to describe the polydipsia and polyuria that are a clinical hallmark of that disease [4]. Polyuria was recognized in China in the third century but not recorded in writing as "sweet urine" until the seventh century [3,6]. An Indian physician described a sign of diabetes as "honey urine" in the fifth century [7]. The Arabian physician, Avicenna (980–1027 A.D.) was the first to describe complications of diabetes including gangrene [8]. Pathologic descriptions of glycosuria began with Paracelsus (1493–1541) and Willis (1621–1675) who also added the Latin word for honey, "mellitus", to the name, diabetes [9]. Johan Frank differentiated diabetes mellitus from diabetes insipidus in 1794 [9]. The French chemist Chevreul (1797–1889) showed that the sugar that spilled into the urine in diabetics was actually glucose [10]. While suggestions had been made that sugar may be elevated in the blood in patients with diabetes, it was Chauveau in 1856 who established that hyperglycemia was a hallmark of diabetes and critical in its diagnosis [11].

1.1.2. Clinical diabetes prior to insulin

The pancreas was first implicated in clinical diabetes in 1788 following an autopsy by Cawley of a 34 year old who had diabetes secondary to chronic pancreatitis [12]. Several reports of diabetes secondary to pancreatitis then followed by Bright in 1831, Bouchardat in 1875, and Lancereaux in 1877 [7,13,14]. Paul Langerhans first identified pancreatic islets during his thesis as a medical student in 1869 by careful evaluation of histologic sections of the pancreas observing isolated nests of small, clear cells scattered in the pancreas that he speculated might by lymph tissue [15]. The relationship of his findings to diabetes was not appreciated until suggested by Schafer in 1895 [16]. It was Opie in 1901 who first described hyaline degeneration of the islets in diabetic patients [17]. While Brunner had described the removal of the dog pancreas was followed by polyuria and polydipsia in 1682 [18], its importance was not understood until Mering and Minkowski reported a series of dog pancreatectomies in 1889 that defined diabetes as an absence of some function of the pancreas [19]. Then Laguesse in 1893

first suggested that the "Islets of Langerhans" produced an internal secretion that was important in the prevention of diabetes. His contribution followed Claude Bernard's suggestion that the physiologic levels of glucose might be controlled by some secretion from the pancreas [20]. The specific name "Insulin" was first coined by Schafer in 1916 to name this hormone from the Latin *insula* meaning island, tying it forever to islets [1]. Later, Macleod used the name insulin to describe the hormone discovered in his laboratory in 1921 by Banting and Best [21], who successfully treated the first diabetic patients with insulin from extracts of duct-ligated pancreases [22,23]. The importance of the discovery and use of insulin to treat diabetes is emphasized by the 1898 study of Fitz and Joslin who treated 172 diabetics prior to insulin therapy and documented that 81% of the deaths from diabetes were due to coma, presumably from ketoacidosis [24]. Joslin also reported in 1917 that of 59 patients diagnosed with Type 1 diabetes before the age of 10 years, 38 or 64% had died within an average of 1.4 years after diagnosis [25]. Prior to insulin therapy, the only "treatment" for diabetes was starvation on an "Eskimo diet" of less than 500 cal per day, bringing patients to severe states of wasting prior to death most often from diabetic coma, as well as from infectious diseases and tuberculosis. As shown in Fig. 1, there were dramatic, lifesaving results achieved with insulin injections as a real treatment for diabetes. Death by coma dropped significantly very early after insulin was discovered and delivered as a drug. Today the death from diabetic coma is less than 5%.

1.1.3. Pancreatic tissue implants

With the appreciation of diabetes being related to the pancreas in the later years of the 1800's, the first attempts at transplanting pancreas began, well before the discovery of insulin, driven by the futility of trying to treat diabetes by starvation. In 1893, P. Watson Williams at the Bristol Royal Infirmary implanted 3 pieces of sheep pancreas subcutaneously into a 15 year old boy, who died of coma three days later with the removal of the graft only showing fibrous stroma [26]. Ssobolew had previously been credited with performing the first pieces of pancreas implants in 1902 [27]. In 1924, Pybus reported the results of subcutaneous implants of human pancreas slices in two patients with diabetes with one patient showing a decrease in glycosuria prior to rejection of the implants [28]. Luisada in 1927 implanted pieces of duct-ligated baboon pancreas into two young human diabetics under the tunica vaginalis, but without an observed effect [29]. Additional pancreatic auto-transplants in dogs followed after Minkowski in 1893 auto-transplanted vascularized uncinate lobes in the subcutaneous site and maintained the animals without diabetes after residual pancreatectomy was performed. Removal of the autografts now resulted in a return to the diabetic state in the recipients [30–32]. These autograft results showed that one could not only avoid the problems of allograft or xenograft rejection, but also eliminate the problems of acinar cell necrosis by previous duct ligation. Pancreatic autografts were also accomplished in the spleen and liver with partial success. Pratt and Murphy in 1913 reported a series of splenic auto-transplants in dogs using vascularized uncinate lobes with long-term success, while chopped pancreatic pieces underwent rapid necrosis after implant [33]. It became clear from these early transplant studies that vascularization was critical for islet function and that exocrine pancreas inclusion in the graft led to necrosis that also resulted in islet graft loss. This understanding led to the use of duct-ligated pancreas that were allowed to remain in the donor after ligation until the loss of exocrine tissue was documented. Then the auto-transplant could be implanted without the acinar cell triggered necrosis with ongoing islet function as demonstrated by Brancati in 1929 in dogs [34]. Allen in 1922 showed that the survival of islet tissue in the implanted vascular pedical only survived and functioned after the transection of the vascular pedicle if the implants had received new vessels from the surrounding tissues [35]. It was not clear at that time what permitted the islets to become revascularized. Rundles and Swan have been credited erroneously as achieving the first successful islet transplant in 1957. But, in fact, they implanted an intramuscular auto-transplant of duct-ligated dog pancreas that did function, but these were not isolated islets [36].

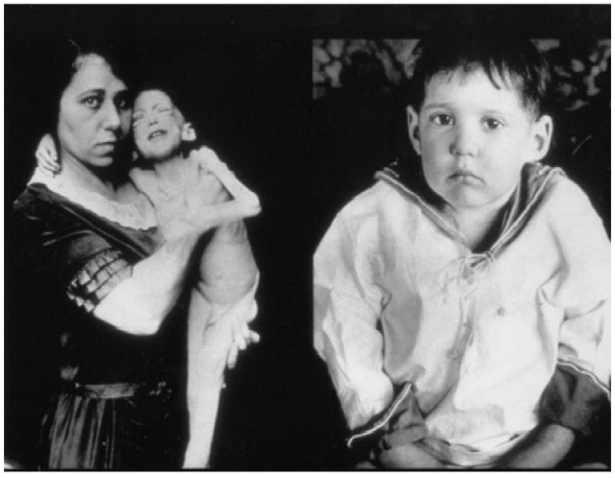

Fig. 1. Starvation as a treatment for diabetes. "J.L." Kentucky child prior to and 1 year after insulin treatment. *Eli Lilly Archives.*

1.1.4. Fetal islet implants

With all of these problems with the exocrine pancreas interfering with the development of islet transplantation, attention turned to the use of fetal or neonatal islet tissue to separate the islets for implant studies. The large endocrine content of fetal pancreas had been recognized by Fichera who, in 1928, transplanted pancreatic tissues from three human fetuses (two of 6 months, and one of 8 months) into three sites into an 18 year old diabetic. But, there was no reduction in the insulin requirement followed by death of the recipient in diabetic coma [29]. Murrell in 1966 had shown that fetal islets develop earlier than the exocrine pancreas and could synthesize insulin so selection of the right fetal age could provide pancreatic implants of relatively pure islet tissue [37,38]. Hegre confirmed this work in 1970 and demonstrated the ability to transplant the cultured fetal islet tissues successfully back to diabetic rodent mothers [39] without immunosuppression. He continued in a series of studies from 1970 to 1976 to demonstrate that fetal islets had a high growth potential both in vitro and in vivo and could successfully reverse diabetes in rodent models [40–43]. Thus, a relatively pure islet source could now be implanted without the complications of the acinar tissue destroying it. But, it also was rapidly rejected when implanted without immunosuppression as an allograft or xenograft. So the question remained as whether fetal or neonatal islet tissue could become a practical tissue for use in treating diabetes.

1.1.5. Immuno-privileged sites

So it was known by then that islet tissue from allograft and xenograft donors was destroyed by an immune and inflammatory response in the matter of days post implant. Having a relatively pure source of fetal and neonatal islet tissue, the attention turned to testing it in immune-privileged sites such as the anterior chamber of the eye, the testes, and the hamster cheek pouch, to demonstrate the feasibility of implanting islets. Browning and Resnik in 1951 showed that mouse fetal pancreatic pieces injected in the anterior chamber of the eye survived longer than adult pancreatic pieces while fetal or neonatal pancreas pieces implanted into the vitreous portion of the eye, the spleen, or subcutaneously were destroyed and did not alter hyperglycemia in the diabetic recipients [44]. The anterior chamber of the eye was shown to be a protector of islet tissues in several additional implant studies. Coupland in 1960 grafted fetal rodent pancreas pieces into the anterior chamber of the eye resulting in acinar loss within 14 days followed by ductal epithelial cells inducing an enlarging islet mass. After one year, these rodent implants had become large masses of

pure islet tissue in the anterior chambers of the eye [45]. Similarly, the testes was shown to protect islet implants by Gonet in 1960 and 1965 implanting fetal pancreas in the testes resulting in the elimination of alloxan induced diabetes and forming intra-testicular masses of islet tissue [46,47]. House in 1958 used neonatal pancreas pieces implanted into the hamster cheek pouch, another immune-privileged site, showing reversal of alloxan diabetes in two weeks and later showed that the neonatal graft survival was dependent upon the age of the donor with engrafted islet survival for 53 days [48,49].

1.1.6. Islet implants

While these fetal and neonatal islet implant results demonstrated the potential of islet transplantation, and while their implantation in immune-privileged sites showed that they could be protected from immune destruction without immunosuppression, there was no practical way at that time to obtain adult islets and no way to protect them from immune destruction outside of these immune-privileged sites. Then, in 1970, Younozai, Sorenson, and Lindall were able to reduce the diabetic state using difficult to obtain adult rodent islets that were implanted successfully into the peritoneal cavity of rodent diabetic recipients [50]. In the same year, Reemstma transplanted fish islets into the anterior chamber of the eye, the muscle, and also into the peritoneal cavity with these islets encased within a Millipore chamber resulting in transient normalization of blood glucose [51]. The battle to obtain isolated adult islets from the pancreas began with a laborious microdissection technique that was only marginally successful, but could best be done using Ob/Ob mice that had very large islets as described by Hellerstrom [52]. Then Moskalewski in 1965 first used a crude collagenase mixture to digest some islets out of a chopped guinea pig pancreas [53]. Lacy & Kostianovsky in 1967 improved the yield of islets by first injecting the enzymes into the duct system before chopping the pancreas into pieces and digesting it. This significantly increased the number of intact islets that were partially purified by the use of a sucrose gradient [54]. Sorenson improved the gradient in 1968 by the use of Ficoll [55] that was modified by Scharp in 1973 [56] providing much purer islets without the sucrose toxicity. Then, in 1972 Ballinger and Lacy using digested and purified islets ameliorated the diabetic state in rodents by implants in muscle and the peritoneum. Removal of the grafts returned the recipients to a full diabetic state setting the stage to obtain sufficient numbers of islets to advance islet transplantation [57]. These results were independently confirmed by Barker and Naji [58]. These partially functional adult islet graft results in the peritoneal cavity were able to be advanced to the complete and long term elimination of diabetes in rodents by injecting the purified islets into the portal vein where they lodged in the liver, became vascularized, and permitted long term reversal of diabetes by Kemp, Knight, Scharp, Ballinger, and Lacy in 1974 [59]. These pivotal studies advanced from rodents, to dogs, and primates through the years. Then, in 1989, the first human patient with diabetes was successfully implanted with purified human islets implanted into the portal vein resulting in the elimination of the insulin requirement for 37 days by the Scharp and Lacy team [60]. Several additional islet implants for many months of partial function were demonstrated in follow-up studies [61]. It is critical to note that all of the successful clinical islet implants that have followed to date have required immunosuppression for graft acceptance.

It is generally recognized that the complications of full immunosuppression required for islet transplant survival performed in order to prevent the long term diabetic complications are simply not an acceptable risk/benefit trade off for patients with diabetes, thus currently limiting its application as a clinical therapy. The acceptable situations for clinical islet transplants with immunosuppression are clearly in those diabetic recipients requiring a kidney transplant requiring immunosuppression. Then the choice is to transplant a whole pancreas versus an islet transplant with clinical results becoming fairly similar now. There are also some very difficult to control diabetic patients in whom the risks of immunosuppression are less than continuing progression of their complications who should be given the opportunity for an islet or whole pancreas transplant with immunosuppression. The only possible alternative approach to avoid immunosuppression at that time came from rodent transplant success in altering the islets prior to implantation to remove donor antigen presenting cells. This approach was referred to as Immuno-Alteration, as developed by and reviewed by Paul Lacy and others [62–64]. But this approach has not been successfully translated to large animals or humans, to date. Thus, immune-isolation remains the only current approach to permit clinical islet transplantation without immunosuppression.

Now with available human and large animal isolated islets, and their long-term success when implanted into an immune privileged site, it became apparent that some form of an artificially created immune privileged site should be technically possible to achieve. Thus, the interest raised to a new level to develop some sort of islet encapsulation techniques to enable these disease-ending islet implants to be accomplished without requiring the use of these dangerous drugs.

1.2. Concerns for clinical islet encapsulation achieving clinical success

The senior author, David W. Scharp, MD, had the opportunity to publish the first review for Islet Encapsulation nearly 30 years ago collecting the early efforts to develop devices to artificially create an immune-privileged site. He coined the name of “Immuno-Isolation” in the title of that publication to represent an approach to protect the encapsulated islets from autoimmune recurrence as well as allograft and xenograft immune destruction [65]. We now know that there are many other causes of destruction of encapsulated islets that are not immune. These include inadequate oxygenation, acute inflammatory reactions from the surgical implant, build up of proteins followed by macrophages and other cells on the diffusive surface of the implant, encapsulating components lack of biocompatibility and intolerance of islet cells, and many more that are presented in other articles within this review issue. While clinical studies are in progress, the current unfortunate reality 30 years later is that there has only been one encapsulation technology that has been approved for implantation into non-immunosuppressed Type 1 diabetic recipients by any regulatory agency up to the current time. That approval was given under the European Agency of the Drug by the Italian Institute of Health (prot 19382 PRE805) to R.Calafiori. Thus, there remain a number of issues with islet encapsulation that have not been resolved. Before the rest of the review, it is important to have some of the major limitations in mind as one goes through the review in order to put the different results in perspective towards a clinical therapy. Table 1 presents the key overriding concerns that currently limit encapsulated islet implants from achieving the level of a clinical therapy.

1.2.1. Rodent results not translating to large animals and human needs

The first concern is the reality that an overwhelming majority of publications of islet encapsulation utilize in vivo rodent models. While it is understandable the rodent models are the appropriate first entrance to animal studies in terms of feasibility and cost considerations, it is also now clear that rodent models cannot predict success in the majority of encapsulated implants in large animal models. As previously

Table 1
Concerns limiting encapsulated islets achieving the level of a clinical therapy.

The results of most of the encapsulated islet studies in rodents translate poorly to large animals and humans with diabetes.
There is a universal acute loss of encapsulated islets following implantation most likely resulting from acute islet hypoxia.
There are residual requirements for immunosuppression in some encapsulated islet studies that need to be eliminated.
There is a risk of transplantation antigen sensitization to recipients of encapsulated islets without immunosuppression.
The insufficient quantities of human islets currently available prohibits islet transplantation from becoming a clinical therapy.

discussed [66], if the encapsulation technique fails in rodents, it will almost never succeed in larger animals. If the encapsulation techniques are successful in rodent models, it still has a great risk that it will still fail in larger animals due to their more robust and multi-tiered responses to devices and to allograft and xenograft tissues. Thus, clinically relevant studies must focus on large animal models as early as possible after rodent model success. These highly published failures of the translation of rodent model success of encapsulated islets to larger animals make it very difficult to assume that the regulatory agencies will readily accept clinically relevant studies to proceed directly from rodents to human implants. Large animal studies of islet encapsulation are critical to enable any judgment to be made of the potential risks and success in human applications. This reality needs to be in the forefront of study planning and development of clinically relevant approaches.

1.2.2. Acute islet loss post-implant

A second concern is that there is nearly always an acute loss of a significant percentage of the encapsulated islet mass within the first week or two of the implant of encapsulated islets in animals, small or large. The current focus on this problem is on oxygenation limitations to the islets immediately after implant, assuming that the integrity of the capsule remains to afford immune-protection. But there are biocompatibility issues as well that make this problem worse. Details of these efforts are summarized in other sections of this review issue.

1.2.3. Requirement for immunosuppression

A third concern in large animal and human encapsulated islet studies is an ongoing requirement for immunosuppression in many studies and in plans for clinical trials. Full immunosuppression simply cannot be a requirement for encapsulated islet implants as a clinical therapy due to the obvious risks of infection and increased cancer risks. The risk/benefit ratio becomes especially important in a treatment modality for diabetes designed to prevent ongoing complications over long periods of time. While it is quite appropriate to use immunosuppression for a combined kidney/islet transplant and for highly selected islet only implants, the ongoing use of immunosuppression for an encapsulated islet implant has reached the not acceptable level for clinical therapy consideration. While there may be a benefit in an acute, low dose anti-inflammatory and/or anti-immune medication immediately after implant, longer term treatment suggests that the device approach is not sufficiently protective of the islets after implant and needs further improvements to eliminate these drug requirements. This elimination should ideally be completed before considering clinical trials.

1.2.4. Donor sensitization

A fourth concern is the recognition that the implantation of encapsulated human islet allografts without immunosuppression can and does sensitize the recipients to allograft transplant antigens. While one encapsulated islet study by Calafiore in four Type 1 Diabetes recipients without immunosuppression did not demonstrate any change in the negative HLA-Class I or II antibody levels nor in anti-GAD65 or anti-insulin antibody levels [67] the recent clinical trial without immunosuppression in two Type 1 Diabetic recipients reported in this review by Novocell (Section 2.3.1.2.2) demonstrated early increases in both diabetic and HLA antibodies post-implant in one of the two recipients. An earlier publication of encapsulated human islets implanted in hollow fibers into three non-diabetic, three Type 1 Diabetics, and three Type 2 Diabetics, demonstrated serologic sensitization in 2 of 9 recipients who had been previously sensitized to HLA shared with the encapsulated islets [68]. Thus, there is an important potential risk of donor sensitization that could become critical in those who may need a kidney transplant within a reasonable time after receiving an encapsulated islet implant. Once encapsulated islet implants achieve clinical success, they should eliminate the need for a kidney transplant. But in this time of developing encapsulated islet implants towards clinical success and with the need for multiple islet donors per recipient, this potential risk of transplant antigen sensitization remains and needs to be clearly considered in patient selection for these trials. These kinds of clinical considerations are further discussed in the clinical trials section of this review issue.

1.2.5. Insufficient islets for a clinical therapy

A final initial concern is the critical lack of sufficient quantities of human islets to achieve even a small suggestion of a clinical product for the treatment of diabetes, in either Type 1 or Type 2 patients. There are under 10,000 organ donors in the USA per year now and over 1.2 million people with Type 1 Diabetes, 25 million with Type 2 Diabetes, and over 75 million with Pre-Diabetes in the USA alone. Global estimates of diabetes are that the current 330 million diabetics will increase to over 500 million by the year 2030 [69,70]. A critical requirement to develop a clinical encapsulated islet product to treat these numbers of patients requires a major ability to expand human islets or to use porcine islet xenografts successfully. Encapsulation of islets can help with either allograft or xenograft islets, but very large numbers will have to become available in order to meet the needs of a wide spread clinically relevant encapsulated islet product.

While there are many other current concerns limiting clinical success (Section 7), the following review (Sections 2, 3, & 4) is designed to provide a look at the early history of islet encapsulation as well as its current progress towards a clinical reality. The important role of corporate funding of Islet Encapsulation projects is also added to this review (Section 5). The final portion defines the critical needs that have to be solved to achieve clinical success (Section 7).

2. Structural approaches for islet encapsulation

Islet encapsulation structural approaches fall in four primary categories: a) macro-devices holding islets within a major structure with selective the permeability component on the perimeter of the device; b) micro-devices encapsulating individual islets or small groups of islets within spherically shaped capsules containing the islets with the capsule providing the permselective function; c) conformal coatings placing a thin covering around individual islets that also is permselective; and d) nanoencapsulation and layer by layer coatings placing a thin film of protection on each islet.

2.1. Macro-devices

Macro-devices are of three major types: a) Extravascular Diffusion Devices most commonly placed outside the vasculature usually in the peritoneal cavity, an omental pocket, or a subcutaneous site; b) Intravascular Diffusion Devices placed into an arterial vessel via a vascular connection; and c) Intravascular Ultrafiltration Devices placed with a direct connection of both ends into an artery but with a connection on the outside of the ultrafiltration membrane of the device for insulin delivery into a vein.

2.1.1. Extravascular Diffusion Devices

2.1.1.1. Early approaches. While Bisceglie in 1933 implanted insulinoma tissues in a permselective membrane to determine the loss of vasculature on survival of implanted tissues, he did not appreciate the therapeutic potential of this approach [71]. The credit for originally developing the Extravascular Diffusion Device should go to G.H. Algire and his colleagues, R.T. Prehn and J.M. Weaver, who developed the technology in order to study both the cellular mechanisms of tissue rejection and tumor growth in a series of publications from 1948–1959 [72–81]. While not conceived as a therapeutic immune-isolation device for enabling implant survival against the host immune responses, their studies on mechanisms of rejection firmly established this approach as an ideal device for such implants [74,76]. In so doing, they discovered the problems of cell survival within these devices by creating a potential

space for the cells within flat membranes, the problems of biocompatibility and the encapsulated destructive result from membrane overgrowth, and the delay in rejection by membrane encapsulation. They clearly demonstrated that host cell exclusion permits allograft cell survival and that host cell exclusion itself may not protect xenograft survival due to antibody passage. Their work established how these devices could artificially create an immune-privileged site for islet implantation and opened the door to Islet Encapsulation studies that follow their early investigations. The Millipore Corporation provided their own commercial product for cell encapsulation designed on the Algire device, but unfortunately placed the membranes on the outside of the enclosing ring creating a much large tissue chamber that created a major diffusion challenge for those using it for islets [83]. Islet functional survival was limited to only weeks in most studies [51,82–87] or even less in others [88–93] with fibroblastic overgrowth the primary problem. Theodorou and Howell reviewed insulin diffusion kinetics required for these devices and membranes [94]. Helmke showed benefit by using a different nitrocellulose membrane [95] and Weber replaced the membranes with cupraphane bags showing improved survival [85]. In 1980, Valente implanted encapsulated human islet allografts in 13 patients with diabetes with two patients transiently off insulin post-implant using the Millipore chambers [86]. Attempting to reduce the fibroblastic response to the device, two groups bonded collagenase to the outside of the membranes that eliminated the fibrous coating directly on the membranes but only succeeded in the fibrous membrane forming a short distance away that still readily reduced diffusion [96,97] producing a result similar to that seen from implanting silicon tubing.

A second type of Extravascular Diffusion Device came on the scene from Amicon that developed hollow fibers for renal dialysis that started the approaches for their use in the Intravascular Diffusion Device to be discussed in the next section. Knazek in 1972 clearly demonstrated the use of these fibers to grow and maintain different types of cells in vitro [98] with Chick showing their utility for culturing islets [99]. Lanza and Chick of Biohybrid used these hollow fibers and ones with larger diameters for implanting islets encapsulated inside the fibers as an Extravascular Diffusion Device with some reversal of diabetes from the graft function in different species in a series of studies [100–107]. But, fiber fragility with bending and breaking and loss of islet mass due to diffusion restrictions in the center of the 600 μm diameter devices limited the duration of these studies. Cytotherapeutics developed another hollow fiber approach using different coextruded tubular hollow membranes to encapsulate islets as extravascular implants. These fiber extravascular devices had good diffusion characteristics and good results in diabetic rodents [108,109]. Additional studies were funded by Inserm in France using this type of hollow fiber technology with functional results in rodents, but large animal results were not published [110–112].

These rodent results led to an FDA approved, non-therapeutic safety clinical trial under an Investigator IND in 1994 by Scharp. Cryopreserved human islets from a single donor were implanted within subcutaneous devices into 9 human recipients (three without diabetes, three with Type 1 Diabetes, and three with Type 2 Diabetes) [113]. Explanted capsules showed minimal implant site attachment and viable islets within the devices from all three types of recipients as determined by in vitro glucose stimulated insulin release. The encapsulated islets recovered from patients without diabetes, readily released insulin in response to glucose alone as well as to glucose plus theophyline. But, interestingly, the encapsulated islets retrieved from both types of diabetics failed to release insulin in response to glucose alone, but did release insulin in response to glucose plus theophylline. Examination of their oral glucose tolerance results, showed that both types of diabetics maintained their average blood glucose levels above 200–250 mg/dl, that are the levels shown to reduce human islets ability to respond to glucose challenge. These findings also define another challenge to implanting devices with islet mass insufficient to achieve normal blood glucose levels post implant, as their insulin release will be inhibited by the higher blood glucose levels in this range. Human islet transplants under full immunosuppression demonstrate both cellular and serological sensitization to donor antigens that can be involved in the implanted donor islets loss of function, especially with donor sensitization prior to implant [114]. The analysis of the 9 recipients in this study of encapsulated islets showed that all were protected against cellular sensitization but 2 of the 9 developed serologic sensitization to the donor HLA [68]. This finding raises concerns that donor sensitization may become a problem in encapsulated human islet implants without the use of immunosuppression. Further development of this approach with this type of device was curtailed for diabetes due to the low packing density limitations that would require very large lengths of such a device for clinical therapeutic relevance.

2.1.1.2. Current approaches. There are currently three Extravascular Diffusion Devices under development towards clinical application described below that are also discussed in Section 5.

2.1.1.2.1. "Theracyte Approach". The first was initiated by Baxter Healthcare in the late 1990's as a planar device of two composite membranes sealed at all sides with a loading port or ports. The outside of the device is designed for strength and to encourage host tissue to incorporate into its outer portions. The other sections are a Teflon-based membrane to encourage capillary ingrowth and a hydrogel semipermeable membrane for allograft immune protection. The initial rodent studies were promising with functional outcomes [115–117] followed by studies adding implant improvements and demonstrations for delivering cell-based products [118–120]. Since this device by design, "grew" into the host by its reactivity, it would be difficult to remove and replace. Neocrin was challenged with converting this device to a "flush and reload" device (Section 4.2), but was unable to successful complete this transition (Scharp, unpublished data). With reduction in the Baxter Gene Therapy project, the device was sold to its startup company and became the Theracyte device. While continuing with publications from several different researchers, it also has been tested by Living Cell Technologies (LCT), Betalogics of Janssen Pharmaceuticals, and Viacyte as well as a number of academic investigators (Section 5). These studies continue and are in different modifications to attempt to create a clinically relevant device, since the original patent has lapsed. The primary current interest in these modifications are driven by those wanting to utilize it for encapsulating human embryonic stem cell (ESC's) derived islet tissue since it is sufficiently strong to keep any of these cells from escaping from the device. The concern for preventing encapsulated cell escape is that there may be very small numbers of non-differentiated ESC's within these islet preparations that can cause benign tumors to form if allograft rejection does not destroy the cells that might escape a device. The ability to propose less robust encapsulation devices or coatings for islets differentiated from ESC's may have a higher hurdle to cross with the FDA to permit clinical safety testing, let alone efficacy testing. However, this question has been recently reviewed by Aznar and Gomez who suggest that four clinical trials utilizing human embryonic stem cells in vivo have been approved by the FDA with 2 listed in ClinicalTrials.gov. Of these two, one was Geron without cell encapsulation, and the other or Advanced Cell Technology in the United Kingdom [121]. While there was a presentation of the first clinical results of the Geron Clinical Trial at a City of Hope sponsored stem cell meeting in 2012, Geron closed the trial shortly thereafter as well as the program without evidence so far of a published report on their injecting unencapsulated human ESC-derived spinal cells for the clinical treatment of spinal cord crush injury. Due to the lack of a large animal model, this trial was approved for a mouse to human application. Yet, these efforts remain controversial [121–125].

2.1.1.2.2. "Islet Sheet Device". A second planar flat sheet device which also begun in the later 1990's utilizes supported alginate sheets containing islets within that was designed to not grow into the host but instead become an "all in/all out" device (see Section 4.2). This type of device can readily be removed and replaced as required by the duration of

the encapsulated islet survival. While rodent studies have demonstrated good function in the intraperitoneal site, the implantation into larger animals has been more difficult to achieve success [126]. More recently, human islets were encapsulated within their islet sheets and kept both in vitro and in rats with demonstrated islet survival after explant [127]. Their group has also published their expected requirements for a successful encapsulated islet device for clinical application [128]. Additional details about Islet Sheet Medical are in Section 5.

2.1.1.2.3. "Beta-O_2 Devices". Beta-O_2 has focused on a critical problem of Extravascular Diffusion Devices that is the insufficient oxygen available for encapsulated islets in macro-devices after implantation that results in early large islet losses. They have explored several different approaches from oxygen generation to the direct supply of oxygen gas to the device. Their first publication describes a combinational device containing an empty portion of the device connected to tubing that permitted daily replacement of oxygen through external tubing. The islet-containing portion of the device was connected to the oxygen portion of the device by a silicone membrane on one side and a unique membrane connected to the surface for the interface to the recipient for insulin release. This device returned diabetic rats back to normal blood glucose values for several months [129]. They have advanced their development to large animals utilizing diabetic mini-pigs. Their preliminary studies demonstrate that their encapsulated islet allografts were kept quite functional for significant times within their double-chambered device connected to subcutaneous ports by which oxygen was delivered to the device by daily injection [130,131]. While the current approach may be cumbersome with external oxygen sources, their clinical trial plans only include a daily injection of oxygen into their device for a more acceptable method of oxygenizing their encapsulated islets. With ongoing success in large animals, Beta-O_2 may be able to obtain clinical approval to initiate clinical trials in the near future (Section 5).

2.1.2. Intravascular Diffusion Devices

2.1.2.1. Early approaches. After the Knazek demonstration that the Amicon multiple fiber device could maintain islets in vitro with perfusion of the fibers with culture media [98], the potential for placing these hollow tubes with islets into the blood stream soon followed essentially in parallel with their use as Extravascular Diffusion Devices as discussed above. Chick's group loaded islets on the outside of the small bored hollow fibers within the devices and connected them into the blood stream of mice and rats with flow down the small tubes. With heparin these device implants resulted in normoglycemia in several diabetic recipients [99,132]. Fairly quickly, Tze, Sun, and Orsetti repeated this success [133–135]. But, the problem with the small diameter hollow fibers continued to be coagulation of the devices requiring high doses of systemic heparin. The device–vessel interconnection was also a problem that threatened this approach from bleeding to partial arterial disconnection and sudden death. Tze replaced the multiple tube device with a single bore Amicon fiber reducing but not eliminating the clotting challenge [136,137]. Sun followed this single bore approach and the heparin problem by bonding heparin to the internal surface of fiber but only delayed clotting for a week [138]. In 1977, the Scharp group formed a tube from a Nucelopore flat sheet membrane surrounded by a larger tubular islet compartment and implanted the encapsulated rat islets into the carotid arteries of heparinized diabetic dogs that normalized blood glucose levels for a time [139]. Then a series of studies and product development in the 1990's by Biohybrid and W.R. Grace (Section 5) resulted in the best demonstration of long-term islet function in this type of intravascular device with several months of islet xenograft function in diabetic dogs without any immunosuppression [101–103,140–142]. While this "hockey puck" design of a large bore tube coiled within a circular device was successful in large animal islet xenografts without immunosuppression for many months, the device-vascular interface proved its loss. The FDA's approval process was actually in process to determine the ability to initiate a clinical trial with this technology when the last long term surviving dogs abruptly died of acute blood loss due to a sudden breakdown of the carotid artery/device connections. This dramatic result caused the clinical trial to be denied and this corporately funded program to be closed.

Separately, Calafiore in 1991 using a PTFE based hollow fiber device placed it between the iliac artery and the contralateral iliac vein and demonstrated porcine and human islet xenografts within alginate micro-capsules with partial function for a couple of months in diabetic dogs without immunosuppression [143,144]. He also implanted human islet allografts with islets in the vascular prosthesis with partial results in 1992 [145]. But ongoing research with Intravascular Diffusion Devices has remained on hold since the early 1990's.

2.1.2.2. Current approaches. With significant advances in connecting small vessels with new materials developed into new vascular surgery products and with other more protected and smaller arteries available for potential implant, it is unfortunate that these kinds of intravascular approaches for encapsulated islet treatment have not been successfully re-visited. But then in 2008, there was a report by Prochorov from the Belarus State Medical University in Minsk, published in Advances in Medical Sciences of 19 patients with Type 1 Diabetes being implanted with third trimester fetal rabbit islets into arterial-venous fistulas with these xenograft islets contained in micro-porous, macro-capsule nylon devices. They report reductions in insulin requirements, both hyper- and hypoglycemic comas, and fructosamine levels, along with increases in C-peptide and insulin in 14 of the 19 recipients out to two years without immunosuppression [146]. Regardless of how one may interpret these specific result details or the lack of them, this study raises the question of whether new materials with new intravascular approaches may yield new results that could lead to a clinically relevant Intravascular Diffusion Device.

2.1.3. Intravascular Ultrafiltration Devices

2.1.3.1. Early approaches. All of the Extravascular Diffusion Devices reported here have the commonality of their being based on simple diffusion restrictions as the method for the inward flux of nutrients, glucose and amino acid for insulin stimulation, vitamins, minerals, and very importantly oxygen for islet survival and function along with the outward flux of insulin and all of the waste products. The design of these devices impacts their functional capabilities since the implant function is based on simple diffusion principles. Some years ago, Sparks published mathematical models to predict diffusional device requirements for function [147]. The following equation is a simplistic representation of his findings:

Formula 1

$$\frac{dp_2}{dt} = \frac{MDA \times DC \times \Delta P}{GV \times MT}.$$

This equation states that the appearance of the product (P_2) or insulin in the blood (dp_2/dt) is directly proportional to the effective membrane diffusional area (MDA), to the diffusion characteristics (DC = lumped mass transfer coefficients, membrane coefficient, luminal fluid flows, and others), and to the difference in product concentrations inside and outside the device (ΔP = P2-P1) while inversely proportional to the chamber volume holding the graft or islets (GV) and the membrane thickness (MT). For example, the effective membrane diffusional area is critical depending upon device design since one may want to keep that diffusional area as small as possible in situations where the membrane may be reacting to the host. But, in doing so, one reduces the diffusional characteristics to all factors requiring transport to the inside and to the outside of the device. Also, protein deposition on the surface of the membranes after implant can effectively reduce effective diffusion area. The difference in insulin from inside to outside is also critical for the robust release of insulin outside of the

device. Yet, there is negative feedback inhibition of insulin production to islets when insulin concentrations are large that can occur in the device with poor kinetics [148]. For the inverse factors to consider, it is critical to understand that the islet chamber compartment (GV) needs to be as small as possible so as to not reduce the diffusion time for insulin release. If one increases the chamber volume by a factor of 10, the increase in the appearance of insulin outside the device will increase from a few minutes to several hours. Even if an enlarged chamber volume causes a delay in insulin release of 20–30 min, the likely result is that the insulin release will peak sufficiently late to be out of synch with the glucose levels causing hypoglycemia after meals [Scharp, unpublished results]. The membrane thickness factor is important and may not only include the actual membrane itself on the outside of the device but also other separating membranes and distances between them that have to be considered in the calculations. Layers of cells attracted to the outside membrane also can drastically reduce diffusion by increasing an effective membrane layer. More recent mathematical evaluations relating to designing diffusional devices have been published by Duong [149–152].

The most critical factor limiting insulin production and release in diffusion-based devices is the fact that oxygen has very low solubility in water at body temperatures. Clark Colton has published the details of these oxygen requirements in the past and again in this issue [153–155]. Using the diffusion equation above for oxygen appearance within the device, the ΔP from outside to inside the device will be very low. There is far more dissolved oxygen in water at room temperature and even more at 10 °C than at 37 °C which is why hemoglobin carries oxygen in the blood and myoglobin captures it in muscle in mammals. Since there is no active oxygen carrying capacity outside or inside the device, the oxygen solubility in water in large part determines the level of oxygen available for encapsulated islets. Utilization of oxygen inside the device by the islets can reduce the internal oxygen level that will drive more oxygen to diffuse in from the outside. Yet, islets cannot produce insulin without sufficient levels of oxygen [156] so there is a fine line required to enable islet function, especially when the device is placed in a low oxygen environment that is the more common place for macro-devices. Normal arterial oxygen is between 75 to 100 mm Hg while venous oxygen runs 30 to 40 mm Hg with tissue levels around 45 mm Hg. Since islets only receive oxygenated blood through afferent arterioles, have a fenestrated endothelial vascular bed, and release oxygenated blood through efferent arterioles with sphincters onward to the acinar components of the pancreas by a portal circulation, they are normally used to functioning at much higher oxygen levels than many other tissues. Encapsulating the islets places them at much lower oxygen levels than normal, reducing their chance of survival and function. Thus, placing islets inside a diffusion device into an arterial blood supply as discussed in the previous section should be very attractive for optimal islet function. Yet, device/vessel interface failure has not only limited success to date but also has reduced interest to revisit improved designs to take advantage of the increased oxygen availability that is so critically needed by implanted islets to function properly.

Moving from a diffusional device to one relying on ultrafiltration will eliminate most of the diffusional restrictions for encapsulated islets just discussed. But, to date, only Reach's group has significantly investigated this approach from 1981 to 1990 [157–161]. The advantage is that the device connection is arterial to arterial with the islets across the permselective membrane from the arterial blood flow like a diffusion device. But the difference with ultrafiltration is that the islet compartment now has its own outlet that connects to a vein thus making the passage and ultrafiltrate flow positive from the artery through the islet compartment to the venous connection. Thus arterial oxygen levels start high and remain much higher than at tissue or venous levels. The ΔP diffusion requirement for insulin release from the device is eliminated as well as the inversely related graft volume effect by eliminating simple diffusion. Thus, in small animal studies, this ultrafiltration device has worked quite well in their hands. The remaining problem for his approach is the potential fouling of the ultrafiltration membrane separating the artery from the islet chamber. Yet, Reach's U-shaped design reduced this risk. Thus, ultrafiltration is another approach that should benefit from the use of newer vascular materials and improved membranes that were not available when this approach was originally developed in the 1990's. Hopefully, someone will begin to explore its benefits in the near future as they eliminate diffusion restrictions.

2.2. Micro-devices

Micro-devices incorporate individual or small clusters of islets into a spherical hydrogel polymer that is formed by different procedures to both contain the islets and provide them permselective protection from host immune attack. With each islet or a very small cluster of islets being the target for containment within each micro-capsule, the devices become far more simple than macro-devices. Diffusional realities are more manageable due to the low volume and spherical shapes as well as their manufacture since they do not require complex steps to complete. Another advantage for micro-capsules is that each encapsulated islet is protected from host immune attack. If there is a small failure rate in forming the micro-capsules properly, only the few islets so affected will be destroyed. In contrast, if there is a small failure of islet encapsulation in a macro-encapsulation device, the entire load of islets is at risk for destruction by the host immune cells since they are contained in a single volume. On the other hand, the initial problem of islet micro-capsules was their relatively large size compared to the islet or small numbers of islets contained within. This problem resulted in a micro-capsule product with the predominant volume made up of the encapsulating hydrogel with only a very small volume being the encapsulated islets. There has been a great deal more research and development as well as publications for micro-capsules than for macro-capsules with many reviews already published [82,162–175].

2.2.1. Alginate based micro-capsules

Alginate or alginic acid is an anionic polyssacharide originating from sea weed and algae that is a linear copolymer that has two units, mannuronate (M) and guluronate (G), that are dispersed through the alginate in different concentrations and in different lengths of repeating units. The stem portions of the seaweed contain high concentrations of G making it stiffer and stronger while the leaf portions contain high concentrations of M making them pliable. Consecutive G units are designed to rapidly bind cations of calcium, barium, and strontium that have increasing binding strengths with content and length of the G blocks. This cation binding crosslinks the alginate with crosslinking density based on concentration. Exposure to citrate will unbind the cations returning the gelled alginate to a liquid. For islet encapsulation, there is not another hydrogel that crosslinks more rapidly than alginate permitting it to crosslink into a gel when dripped into a solution of calcium or one of the other cations. The vast majority of studies have used alginate micro-capsules due to their ease of formation through different types of drop formation approaches resulting in the rapid crosslinking of alginate upon contact with a calcium or barium encapsulating the enclosed islet.

2.2.1.1. Early approaches. The development of micro-capsule formation came out of the chemical industry for many industrial applications initiating in the 1930's by chemically encapsulating different components within small capsules. A major micro-capsule invention in 1952 was carbonless copy paper by NCR Corp placing tiny dots of dye or ink onto the under surface of typing paper which would break due to force from typing or writing on the original, top paper releasing the dye to leave a copy of the original marks on the second paper [176,177]. Today, almost every industry uses some form of micro-encapsulation in a large variety of applications. In the food industry, micro-encapsulation is adapted to many forms, shapes and methods to achieve food protection by this technology. In terms of micro-

encapsulating living cells, the credit should clearly go to Thomas M. S. Chang, PhD. He was born in China but, as an undergraduate at McGill University in Montreal, turned his dormitory room into a laboratory and perfected the formation of the world's first artificial cell encapsulating hemoglobin in a semi-permeable plastic bag that functioned nearly as well as a red blood cell [178,179]. Chang in a Science article suggested in 1964 that encapsulation of living cells could be used to transplant cells into patients to replace lost cells or replace genes that had genetic defects omitting essential gene functions [180–182]. He had two United States Patents accepted in the early 1970's on blood compatible and non-thrombogenic micro-capsules [183,184]. In a review article in 1989, Chang was highlighted as suggesting the potential treatment of a number of disorders that could be eliminated or reduced by the use of encapsulated cell therapy [185]. Then in 1992, he published a review article on the encapsulation of living cells stating the progress made up to that point [186].

But it was the publication of Lim and Sun in 1980 that focused the diabetes community to begin to accept the concept that islet encapsulation could provide a potential treatment for this disease using microencapsulated islets to create an artificial immune-privileged site for islets [187]. There are a great many studies of micro-encapsulation of islets that have followed this beginning. Since other authors in this issue are addressing the different kinds of hydrogels and polymers involved (P. de Vos), the biocompatibility and physic-chemical characteristics of micro-capsules (B.L. Strand), the nature of semipermeable membrane immune protection (T. Wang), technologies involved in cell encapsulation (R.J. Neufield), oxygen requirements for encapsulated cells (C. Colton), and clinical results of encapsulated islets (R. Calafiore), this section will focus on more general considerations of micro-capsules for islets and the advances that have been made.

The hydrogel micro-capsule for islets with the most publications is the use of alginate to encapsulate the islets followed by a poly-amine such as poly-lysine or poly-ornithine thin coating for permselectivity which either becomes the outside coating or is covered by another layer of thin alginate [188–204]. The islets are encapsulated in the alginate by three basic steps: a) mixing the islets with the alginate, 2) forming micro-droplets containing the islets by extrusion by pumping them through a needle, and 3) collecting the micro-droplets with the islets in a calcium, barium, or strontium ion bath that rapidly cross-links the droplet containing the islet before it disintegrates in the collection fluid. A number of recent publications propose changes to this alginate method of islet encapsulation. There are recent results from the Uppsala, Sweden group in 2003 suggesting that the type of alginate used (high G or high M) can adversely affect encapsulated islet survival with high M being more favorable, contrary to earlier publications. In addition, there is clear evidence that the polyamine outer coatings provided for permselectivity are more reactive to the host inflammatory response reducing islet survival compared to alginate surface coatings alone [205,206]. Then in 2006, the de Vos group demonstrated that it is not the type of alginate (G or M) that is the problem, but rather it is the reactivity of the type of polyamine utilized. They found the Poly-L-Lysine is far more optimal than the others in that it is the least reactive to the host [207]. Though these outer polyamine coatings inflammatory stimulation of the host can be reduced by including dexamethasone in the coatings, their requirement for providing permselectivity to the capsules becomes a limitation for clinical therapy [208]. The Weir group at the Joslin had demonstrated that the use of barium crosslinking rather than calcium crosslinking increased the alginate permselectivity sufficiently that there was no need to use the polyamines that cause so much trouble to get islet allograft protection long term in NOD mouse recipients [209]. Many hundreds of publications have shown the implantation into diabetic animal recipients bring excellent results [210–221].

The first method of alginate encapsulation of islets is the simple extrusion method as described which usually results in micro-capsules of 800–1000 μm diameter which is quite large in comparison to the islet. For example, if an average-sized islet with a 150 μm diameter is encapsulated in a 1000 μm diameter micro-capsule, the volume of the micro-capsule is 296 times the volume of the islet. This makes the consideration of transplanting 1 million islets of this size encapsulated in this large of a micro-capsule formidable and even difficult in humans when understanding the transplant volumes involved. This approach has been tried in both dogs and humans in a clinical trial but only with marginal success by Soon-Shiong [222–224]. By comparison, if the same 150 μm diameter islet is encapsulated in a 250 μm diameter conformal coating or minimum volume microcapsule, the volume of the microcapsule is only 4.6 times the volume of the islet. Now one can consider implanting the micro-encapsulated islets in different sites in humans due to the smaller total volumes involved. Several approaches have been developed to reduce the size and volume of these types of alginate microcapsules that are designed to reduce the surface tension of the droplet as it forms on the end of the needle so the drops will come off in smaller sizes. The first was to use coaxial needles pumping the alignate/islet mixture through the inner needle and compressed air in the outer needle to break the formed bubble surface tension by essentially blowing off the droplet at a smaller diameter which is fairly effective to the 300–800 μm size [225–228]. Additional methods to reduce droplet size include placing a charge across the needle to the fluid below creating an electrostatic potential to reduce droplet size that can get to 200–600 μm diameters. Additionally, one can use an airknife to mechanically chop the droplets smaller as they are extruded or use vibration or ultrasonics to reduce the droplet size [229–237]. More recently published in 2011, the use of a micro-fluidic device by the Opera group seems more controllable and perhaps more efficient than the older methods [238].

The challenge with alginate encapsulation in making the capsules smaller is that there is no centralizing force during the droplet formation of the capsule. In fact the short distance from the needle to the calcium bath is sufficiently long enough for gravity to have an effect on the much denser islet in the alginate droplet pulling it to the bottom edge during the drop. This leaves some islets on the edge of the capsule after alginate cross-linking that can be exposed to the host immune system. Besides increasing the alginate concentration significantly that brings it own problems, the only way around the islet edge problem is through a second coating that can clearly increase the size of the encapsulated islets. The potential result of this edge problem was shown by the Weir group at the Joslin by singly and doubly coating adult pig islets and testing for islet function and host responses in mice. They found that the single coated pig islets created a much larger IgG and IgM response in the immune competent, diabetic mice than was created in the diabetic mice receiving the double coated islets. This clearly shows there were partially coated islets available to the mouse immune system. But, in these mice, there was no difference in functional responses between the two groups, even in the presence of the antibodies. The question remains if the more immune competent larger animals that could respond to partially coated islets would have a stronger result on the islet survival and function than was seen in these mice [239].

With the appreciation of the need for small alginate micro-capsules for encapsulating islets, a number of studies began approaching the concept of Minimal Volume Capsules that were led by Calafiore's work. His studies developed several approaches that resulted in much smaller alginate based micro-capsules that had clinical implantation relevance. [240–246]. He also began to investigate adding Sertoli cells to encapsulated islets to determine if their multiple functions may reduce islets sensitiveness to inflammatory and immune reactivity within the capsules [247–252]. While the Sertoli cell additions made a great deal of sense showing protective effects on encapsulated islets, the inability to control the Sertoli cell gene expression and release of its varied potential products in vitro and within the capsules limited the application of this approach towards a clinical therapy. The problem with lack of control of the Sertoli cell gene expression is not in their potential to produce something harmful, but in their unpredictability of uncontrollable

gene expressions that leads to difficulties in reproducing results. This certainly reduces the ability to enter clinical trials, let alone to develop a clinical product. Calafiore also initiated clinical trials of encapsulated human islets using the Minimal Volume Capsules that will be discussed in his clinical section of this issue [199,253,254].

Another novel approach to making the alginate capsules smaller is to reduce the size of the islets being encapsulated and determine if they can function better in this environment than large islets. This work was published in 2010 by the Weir group at Joslin where they took rat islets and dispersed some into single cells followed by re-aggregation and submitted these islet aggregates and intact islets to the same alginate encapsulation. Their results show a clear improvement using the small islet aggregates in terms of oxygen consumption, glucose stimulated insulin release, and reduced tissue necrosis and inflammatory gene expression. In addition, those mice receiving the small islet cell aggregates had an increased reversal of their diabetes. If these smaller islet cell aggregates could also be encapsulated in smaller capsules, it should improve the alginate mass problem even more [255].

Using many of these improvements a growing number of studies have found repeatable success in rodent models of diabetes. But the numbers of implants in large animals have had less success. Work in dogs and pigs has done fairly well as both species are quadrupeds like rodents so the intraperitoneal injection of islet capsules permit them to remain scattered around the cavity [256]. But, when approaching non-human primate studies with intraperitoneal micro-capsules, the results are varied [245,257]. Some studies did well but others completely failed. The clue to this difference comes from the fact that non-human primates are predominantly bipeds and not quadrupeds. If the microcapsules do not readily stick to the recipient's tissues, then they readily fall to the pelvis when the recipient is in the upright position. Now the shape of the non-human primate as well as the human peritoneal cavity when upright resembles a large living conical centrifuge tube with the islets packing together down in the pelvis. With the low oxygen carrying capacity of the peritoneal fluid, a large percentage of these encapsulated islets packed into the pelvis simply perish in non-human primates [Scharp unpublished results]. On the other hand, if the islet microcapsules are rather sticky in nature, they will tend to stick to any tissue in the peritoneal cavity and are thus prevented from falling into the pelvis. Once stuck, these islets are more prone to become vascularized by the host. But, sticky capsule surfaces can also lead to protein deposition and cell overgrowth reducing diffusion and eventual killing of the encapsulated islets. Sun [198] reported an impressive result in non-human primates with excellent long-term results. But to date, no one has reached the same success and duration of function with this approach. Use of an omental pouch can improve the results of intraperitoneal islet implants in the non-human primates, keeping them from settling in the pelvis [258].

2.2.1.2. Current approaches. Many of the micro-capsule approaches above and those below continue in their development. But one approach is in current clinical trials through Living Cell Technologies (LCT) (see Section 5) with their Diabecell product of porcine islets. Briefly, Bob Elliott has published descriptions of developing an alginate based micro-capsule for implanting pig islets and later neonatal pig islets in animals [245,257]. Then, several clinical trials have been done or are in progress. One was in 1995–96 with 6 patients implanted with one recipient having a 9.5 year laproscopic biopsy with viable encapsulated porcine islets reported [201]. A second clinical trial was performed in Moscow in 2008 with 8 patients receiving encapsulated porcine islets without immunosuppression with two of the three receiving 10,000 IEQ/kg body weight off insulin at 6 months [259]. Details will be given in the Calafiore Section of Clinical Trials in this issue for these trials and the others they have in progress.

Another current approach to avoid classic immunosuppression especially in xenograft encapsulated islets is the potential to block co-stimulatory pathways. The Weber group at Emory has recently demonstrated over one year of encapsulated adult pig islet function in barium/alginate microcapsules implanted in NOD mice by providing continuous co-stimulatory pathway blockade with CTLA4-Ig+anti-CD154 mAb. Control encapsulated pig islets without blockade survived 1–2 months. Control unencapsulated adult pig islets without blockade rejected in a week in the NOD mice, but with the blockade without microcapsules functioned for about a month [260,261]. The next question is whether it can be demonstrated in a large animal model. Hopefully this approach will prove better in the large animal studies than did the Immuno-Alteration studies.

2.2.2. Other micro-capsule approaches

2.2.2.1. Agarose. Agarose is the primary component of agar and is a linear polyssacharide derived from the walls of red algae. The other component of agar is agaropectin that is a heterogenous mixture of smaller molecules. Agar exhibits hysteresis melting at 85 °C and solidifying at 32 °C to 40 °C. Agarose itself consists of a repeating unit, agarobiose, that is made of two sugars, D-galactose and galactopyranose that also gels in the cold. Agarose has been used for many years in electrophoresis and is readily available in defined concentrations and purities. To utilize agarose for islet encapsulation, a 5% solution is heated to 40 °C followed by mixing the islets in MEM media with it. Then paraffin oil is added and the container is shaken to emulsify the water/oil mixture. After the emulsion produces the size of droplets needed, the container is placed in ice water continuing the agitation with the drop in temperature gelling the agarose beads component that contain the islets. The solution is washed and the encapsulated islets are picked from the other components and washed. Iwata developed this protocol in 1988 and demonstrated the successful reversal of diabetes in mice with isografts having indefinite survival and NOD allografts surviving for 80 days [262]. While Dupuy had also produced agarose bead encapsulation of islets, he did not perform implants [263]. Due to the limitation of human islets for potential clinical therapy, the Iwata team in 1994 turned to agarose encapsulation of hamster islets into diabetic mice as a xenograft model. They found these encapsulated islets survived to 100 days in mice not pre-exposed to hamster tissues. But, they had to increase the agarose concentration to 7% in order to get encapsulated islet acceptance in mice previously exposed to hamster cells to protect the encapsulated islets from the antibodies. They also determined the diffusion coefficients of solutes like glucose and IgG were inversely related to molecular weight of the solutes using their agarose micro-capsules [264].

Rat islets had been cryopreserved in DMSO and transferred between centers for successful islet implants by Rajotte and Scharp in 1981, [265] as well as the use of cryopreserved human islets for macro-encapsulated implants in human recipients [113]. But, in 2009, Iwata's team first demonstrated the ability to cryopreserve islets already encapsulated in agarose capsules by vitrification. They had developed a vitrification solution, KYO-1, and demonstrated the ability to completely replace aqueous solutions step wise with KYO-1 to 100% with the agarose encapsulated islets within and then freeze them in steps down to −185 °C. Rapid thawing with step-wise dilutions demonstrated successful encapsulated islet implants with long-term survival with only a 50% loss of function when implanted into diabetic recipients. This report is quite a significant demonstration for vitrification of islets especially since they were encapsulated [266]. After these studies, the Iwata team turned to Layer-By-Layer coatings. (Section 2.3).

2.2.2.2. Multi-component alginate encapsulation. Taylor Wang, who is authoring a report on semipermeable membrane immune protection in this issue, came to encapsulated islets via a PhD in solid state physics followed by employment at California Institute of Technology's Jet Propulsion Laboratory (JPL) that led to many shuttle flight studies with micro-gravity bubble technology and other projects. His own NASA Shuttle flight as a payload specialist was on the first operational Space Lab flight, STS-51B/Space Lab 3 mission in 1985 on the Challenger.

With this background on bubbles and other physical properties, he focused his attention when moving to Vanderbilt on developing an improved islet encapsulation technology that would consist of multiple layers and components while attempting to centralize the islets and reduce the capsule size. His first studies in 1997 chose sodium alginate (SA), cellulose sulfate (CS), poly(methylene-co-guanidine) hydrochloride (PMCG), calcium chloride (CC), and sodium chloride (SC) as the optimal combination after searching hundreds of possible combinations. Glucose stimulated insulin release from encapsulated rat islets showed viability that was confirmed with successful mouse implants [267]. The next three publications further define the function of each of the components that improve the strength over alginate and provide significant flexibility in altering the dynamics of the capsules for different purposes [268–270]. The next publication demonstrated the versatility of these coatings by precise manipulations of permeability for different compounds such as IL1, IgG and others [271]. More recently in 2008, he published excellent results of encapsulated dog islet allografts without immunosuppression or anti-inflammatories implanted into the peritoneal cavity in 9/9 diabetic dogs with function out to 214 days. Three of the dogs returned to normoglycemia with a second implant after the first ones began to lose function over time [256]. Only this investigator has significantly studied this unique approach to micro-encapsulated islets.

2.2.2.3. Alginate emulsification encapsulation. A more recent approach for producing smaller alginate microcapsules with islets was recently re-explored, based upon an original study from 1992 by a Canadian group that utilized an emulsion technique to locally release calcium ions to form small alginate micro-capsules [272]. The recent development also from Canada in 2011 and 2012, focused on higher throughputs of encapsulated islets by changes in the buffer system described by Poncelet in the 1992 study in the oil/water emulsion formation. Higher insulin release was described from the encapsulated islets [273]. Formation of alginate encapsulated islet cells using 5% alginate rather than 1.5% alginate for this emulsion approach resulted in improved success in rodent implants in reversing diabetes [274]. This novel approach to islet encapsulation certainly needs additional studies and tests in large animal models of diabetes.

2.3. Conformal coatings

Conformal coatings can be defined as hydrogels that are applied to the surface of an islet or other cell aggregate by methods that form a hydrogel, cross-linked coating by interfacial polymerization. Since the photo-initiator required for the cross-linking is bound to the surface of the islet or aggregate, the polymerization propagates outward for a controlled distance as defined by the crosslinking components and initiation methods that trigger the cross-linking. These are thicker, actually cross-linked hydrogels at 50 to 70 μm as compared to those discussed below under Nanotechnology and Layer-by-Layer Coatings. The Minimal Volume Capsules presented in the Micro-Capsule discussions are made by bulk phase crosslinking alginate with islets or aggregates trapped within the droplet of uncrosslinked alginate that is then secondarily crosslinked when encountering the calcium solution into which it drops. This conformal coating approach utilizes interfacial polymerization.

2.3.1. PEG coatings

2.3.1.1. Early approaches. The polyethylene glycol polymer (PEG) method of interfacial polymerization of cell encapsulation was invented by Jeff Hubbell while at the University of Texas (See Corporate Patents — Neocrin & Novocell). These patents were licensed by Neocrin (Section 5) that collaborated with Hubbell to develop them into an islet encapsulation technology [275–277] and were later licensed to Novocell (Section 5). The PEG Conformal Coating technology covers islets by an interfacial polymerization reaction that is shown in Fig. 2A. For islets or other cell aggregates, the photoinitiator, eosin Y with a cell-attaching component is incubated with the islets resulting in islet-bonded eosin Y on their surfaces. The excess eosin Y is washed away and a solution of co-monomers and accelerants are added in solution to the islets. The primary monomer is PEG triacrylate of the appropriate size along with a second monomer n-vinylpyrrolidone that are mixed with a polymerizing accelerant, triethanolamine. No reaction takes place without the proper laser energy. When it is turned on at the appropriate wavelength the energy forms radicals with sufficient energy to bond the acrylates together as well as the N-vinylpyrrolidone at a sufficient speed from the triethanolamine to form a gel that initiates from the eosin Y at the islet surface. The crosslinking propagates outward from the surface to a controllable distance producing a interfacially crosslinked hydrogel conformally around each islet. By controlling the cross-linking energy properly, one can form spheres with this approach avoiding other non-spherical shapes and tails. Since this is a radical generated cross-linking reaction, appropriate steps have to be made to protect the encapsulated islets from radical damage during their encapsulation or they will be destroyed in the process.

The first encapsulated islet report demonstrated successful xenograft encapsulated islet implants with pig islets into diabetic rodents without immunosuppression [278]. These studies were attempted to be translated to non-human primates but initially failed since these larger animals reacted aggressively to the PEG coatings and equally aggressive to the xenograft tissues [66]. This is another example of rodent model results not being successfully translatable to large animals. After a few years of trying to overcome this large animal restriction, Neocrin closed and transferred its technology to Novocell that, after several years of development, was successful in completing the changes required to demonstrate non-human primate success as shown below. To achieve success in non-human primates, the PEG triacrylate had to be extensively changed to be non-reactive in large animals by itself and improve the ability to bind eosin Y to the islet surface to achieve proper uniformity of the conformal coatings crosslinking density. The speed of the radical cross linking also had to be accelerated to form the proper coating while protecting the islets from radical damage.

2.3.1.2. Current approaches

2.3.1.2.1. Pre-clinical studies in diabetic non-human primates. With Novocell successfully developing PEG encapsulation of islets, a series of pre-clinical studies in non-human primates were completed that were presented at an international meeting under an abstract in 2005 and poster presentation, but otherwise never before published, with the following results reproduced from that presentation [279]. Several preliminary Cynomolgus monkey implants (Scharp, not published) were followed by preliminary baboon implants to establish successful islet encapsulation techniques and dosing requirements. The first results presented below are from the Pilot and Feasibility Studies in diabetic baboons using PEG Conformal Coated Islet Allografts implanted in the subcutaneous site into stable streptozotocin-induced diabetic recipients.

The next figure (Fig. 2B-a) shows the first long term results from diabetic baboon recipient #17938. It starts with initial normal and diabetic periods before receiving the encapsulated islet allografts in the subcutaneous site with only 30 days of low dose cyclosporine at the time of implant. After 30 days post-implant, a level of islet function was reached that permitted insulin independence. After several months of insulin independence, this recipient was put back on insulin and also started on metformin due to a reduced level of implanted islet function. The recipient was then given a second subcutaneous, PEG encapsulated islet implant on Day 424 post-1st implant that again resulted in insulin independence that continued to the end of the study with a total of 570 days of ongoing islet function. The two other recipients of encapsulated islet implants in this Pilot Study that also achieved insulin independence are shown in Fig. 2B-b and only required a single implant

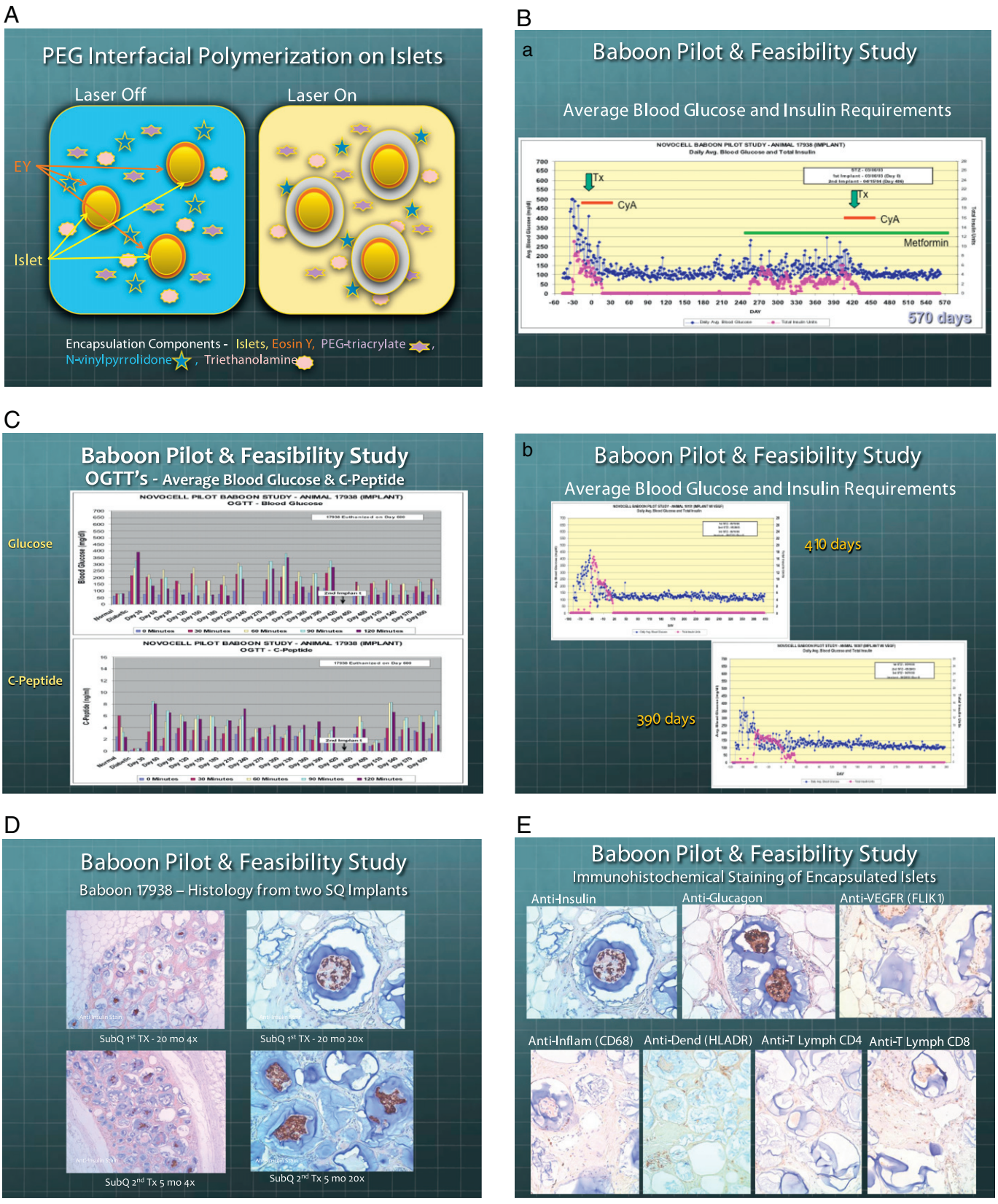

Fig. 2. A. Formation of PEG Conformal Coatings. B-a. Baboon Pilot & Feasibility Study — first long term result with 2 implants. B-b. Baboon Pilot & Feasibility Study — 2nd & 3rd long term results. C. Baboon Pilot & Feasibility Study — OGTT results from Baboon #17938. D. Histology of encapsulated islets 5 and 20 months post-implant in #17938. E. Immunohistochemical stains after 5 and 20 months post-implant in #17938. F. Immunohistochemical histology of #17938 pancreas at termination.

f

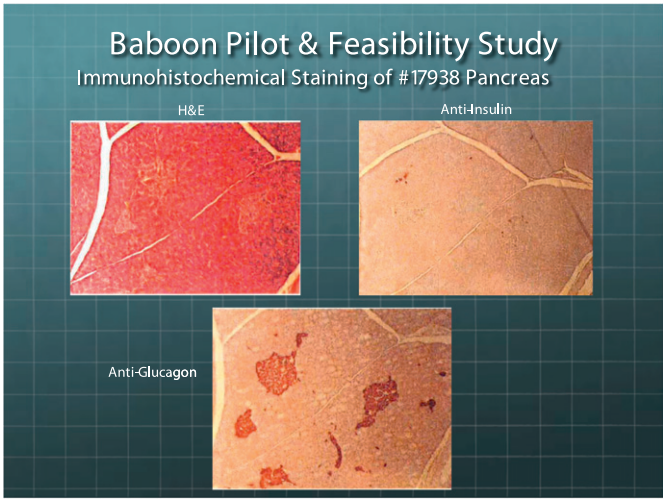

Fig. 2 (*continued*).

for long term insulin independence of 390 and 410 days. Two other recipients of encapsulated islet allografts in this group of 5 for this Pilot and Feasibility Study had partial function but did not achieve insulin independence (Scharp, not published).

The Oral Glucose Tolerance Testing (OGTT) on the first long term Primate #17938 is shown in Fig. 2C. The OGTT was performed monthly and shows normal glucose and C-peptide responses prior to Strep induction of diabetes when glucose response went very high and C-peptide responses nearly disappeared. One month following the first implant, the glucose response was higher than normal as was the C-peptide response. After 8 months, the blood glucose responses to glucose challenge became elevated with reduction in C-peptide that remained for several months. After the second subcutaneous implant, the glucose responses to Oral Glucose Tolerance returned to normal along with the C-peptide responses that lasted in combination for a total of 570 days when the animal was terminated for histology analysis.

The histology from the long term implant recipient #17938 is shown in Fig. 2D. The encapsulated islets had been injected through a 16 gauge long needle into the subcutaneous tissues of the abdomen. Low power histology identifies these long tracts that have some loose fibrous tissue and scarring surrounding the multiple encapsulated islets (data not shown). There are clearly a large number of empty capsules that may represent early loss of islet mass from hypoxia shortly after implantation. But this study was not designed to answer the short term questions. Instead, it focused on long term results. The figure below shows medium and high power views of the implant site stained for insulin showing results from the first implant, 20 months prior to sacrifice, as well as from the second implant site done 5 months prior to sacrifice. As suggested by the previous figures, there is little evidence of major loss of islets throughout the 5–20 month observation period. There appears little difference in the percent of surviving encapsulated islets at 5 months and 20 months, again suggesting the empty capsules lost their encapsulated islets early after the implants. Since the PEG conformal islet technology does not permit the formation of any empty capsules by its method of formation, all of the empty capsules observed had viable islets at the time of implant. The viability of the encapsulated islets at the time of implant was 84%. But, no biopsies were taken prior to three months post-implant. Presumably, most of the encapsulated islets were lost were due to hypoxia within the first two weeks post-implant.

To further evaluate the implant sites, a number of immunohistochemical stains were utilized to identify what level of inflammatory and immune responses were occurring around these long term implants in the subcutaneous sites. Fig. 2E presents these results. In addition to insulin staining that was positive in most surviving islets, glucagon was also confirmed to be present suggesting the entire islet was preserved. In order to identify any ongoing attempts at vascularization, an anti-VEGF receptor stain was used that failed to demonstrate any concentrations of VEGF receptor in the implant site at this 5 and 20 month time. The anti-inflammatory macrophage response was judged by staining with anti-CD68 and failed to show any significant cells remaining. Three stains were used to identify the presence of immune cells. Anti-HLA-DR was used to identify dendritic presenting cells that were seen in the implant site in small scattered groups of small numbers of positive cells. They were not identified at the surface of any encapsulated islets. Anti-CD4 staining was negative, but anti-CD8 staining showed small scattered groups of positive cells throughout the implant site. There were no differences between the 5 month implant sites and the 20 month implant sites with these additional stains. The histology in the other 4 baboons in this Pilot and Feasibility Study showed similar results.

Fig. 2F confirms the adequacy of the streptozotocin protocol used in these studies to specifically eliminate the vast majority of beta cells in the islets to produce a lasting diabetes model. The delay in initiating insulin therapy after strep reduces the chance of spontaneous diabetes reversal. The essence of the protocol was to give the streptozotocin and delay any insulin to the recipient for 7-10 days, as long as ketoacidosis was avoided as can be seen in Fig. 2B-a & B-b. Prior studies had demonstrated that providing insulin too soon after strep injection permitted spontaneous recovery of diabetes due to islet return in these young recipients (Scharp, not published). The H&E staining locates the islets in this series of serial sections. There is very little residual insulin staining even with normal blood glucose levels at the time of sacrifice. There is very heavy glucagon staining throughout this entire study in all islet samples that may relate to the specific anti-glucagon antibody used.

With these positive Pilot & Feasibility Study results completed, a definitive GLP study of diabetic implants for 3 and 6 months duration was performed to develop results suitable to submit to the FDA to request approval to initiate a clinical trial. The design of the study was to produce two groups of 11 diabetic baboons with streptozotocin with the 1st group to be sacrificed at 3 months and the 2nd group sacrificed at 6 months. Within each group of 11, 3 were to be kept as diabetic controls receiving insulin with 8 to be transplanted with PEG conformally coated encapsulated baboon islet allografts. These 8 were evenly divided into those 4 only receiving the encapsulated islets in the subcutaneous site with the 2nd 4 receiving encapsulated islets with the inclusion of a VEGF analog in the injection site at the time of implant. The average viable encapsulated islet implant dosage was 47,077 ± 5140 IEQ/kg/BW per recipient with the encapsulated islet viability percentage prior to implant of 84% meant that 56,044 IEQ/kg/BW was actually implanted. The following results were collected.

As shown in Fig. 3A, the diabetic controls for both group were fairly stable throughout after a couple of initial weeks without insulin to eliminate any spontaneous recurrences of diabetes. But, the daily insulin dose per injection had to be reduced midway in the 3 month group due to hypoglycemic episodes. Both the 3 month and the 6 month groups receiving encapsulated islet allografts in the subcutaneous site had significant reductions in insulin requirement with 3 of the 8 recipients in the 3 month group achieving insulin independence and 2 in the 6 month group as well.

Pre-diabetic OGTT results for all 22 animals had normal blood glucose and C-peptide responses prior to induction of diabetes. In the month following initiation of diabetes, all three groups had significant elevations in blood glucose responses as well as very low C-peptide responses. Note that these results in Fig. 3B are plotted as areas under the curve with the diabetic controls shown in blue columns and the encapsulated islets alone in the red columns with the encapsulated islets with VEGF shown in the green columns. In the 3 month groups post-

implant, the diabetic controls maintained their higher blood glucose levels and their low C-peptide levels.

The values for Hemoglobin A1c (HgbA1c) are presented in Fig. 3C with the diabetic control group shown in lavender, the encapsulated islet implant group shown in red, and the encapsulated islet plus VEGF treatment group shown in blue. It appears that hemoglobin A1c levels in baboons turn over faster than in humans since significant differences are observed in a one month timeframe. While it may not be entirely valid for this study, we chose to adapt human levels of normal HgbA1c levels of <6.0% as normal, 6.0%–6.4% as pre-diabetic and 6.5% and higher as diabetic levels to gain a sense as to where these baboon values would be located. Upon entrance into the study, all three groups in the 3 month study and the 6 month study showed HgbA1c levels between 4.7% and 5.1%, that includes 22 baboons, that we are calling normal values. After one month of diabetes, all 22 baboons showed increased HgbA1c levels to 6.6% to 7.3%, that are clearly in the human diabetic range. In the 3 month group, the diabetic controls dropped to 6.2% and 6.3% for the first two months on a heavier insulin dose, but rose to 7.1% after decreasing the insulin dose. By the second month post-implant of the encapsulated islets, both the islet alone group and the islet plus VEGF group begin to drop their HgbA1c levels from the diabetic range to 4.8% and 5.6% in the third month post-implant. For the 6 month group, the diabetic controls HgbA1c levels go above 7.0% and stay between 7.0% and 7.4% except for month 5 when there was a temporary dip to 6.6%. For the encapsulated islet implant groups, their HgbA1c levels drop to normal levels at 4.8% to 5.8% for the first 4 months, but elevate some for months 5 and 6 to 6.1% to 6.4% that are at pre-diabetic levels. This confirms some of the higher blood glucose levels seen in months 5 and 6 as noted above in some of the recipients. So it appears the HgbA1c levels can be valuable for these kinds of studies in baboons. A recent study in Cynomolgus monkeys found similar hemoglobin A1c results in diabetic and porcine islet xenograft recipients [280].

The histologic findings from the 3 month group of the GLP Study (Fig. 3D) are quite different from those from the Pilot and Feasibility Study at 20 months and 5 months shown above. The 3 month H&E slides show far more recent reactivity than was seen in the long term sites representing more recent recovery from the surgical injection as well as expected more reactivity presumed to relate to the hypoxic loss of islets immediately post-implant. The anti-insulin staining shows a number of viable islets in the site, as well as the anti-glucagon staining. The anti-VEGF receptor staining shows a lot of activity within the implant site at 3 months with linear strings of positively stained cells outside the capsules along some of the capsule surfaces and between capsules. Also note that the encapsulated islet cells show positively stained cells to anti-VEGF receptor both in clusters and in

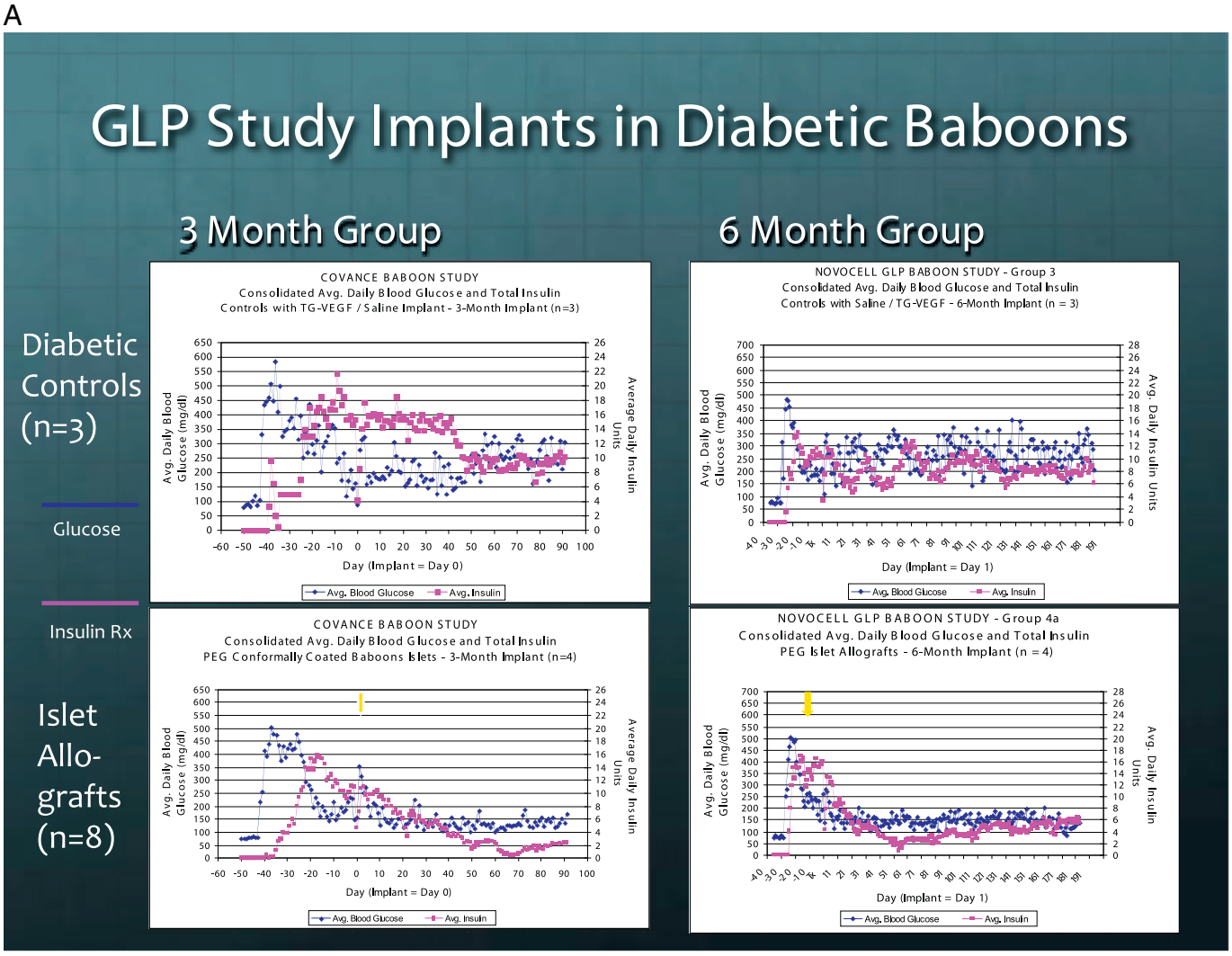

Fig. 3. A. GLP study of PEG conformal coated islet implants in diabetic baboons. B. OGTT glucose & C-peptide responses in GLP Baboon Study. C. Hemoglobin A1c levels in GLP Study Baboons. D-a. 3 month histologic analysis of encapsulated islets in the GLP Study. D-b. 3 month histologic analysis of encapsulated islets in the GLP Study. E-a. 6 month histologic analysis of encapsulated islets in the GLP Study. E-b. 6 month histologic analysis of encapsulated islets in the GLP Study.

B

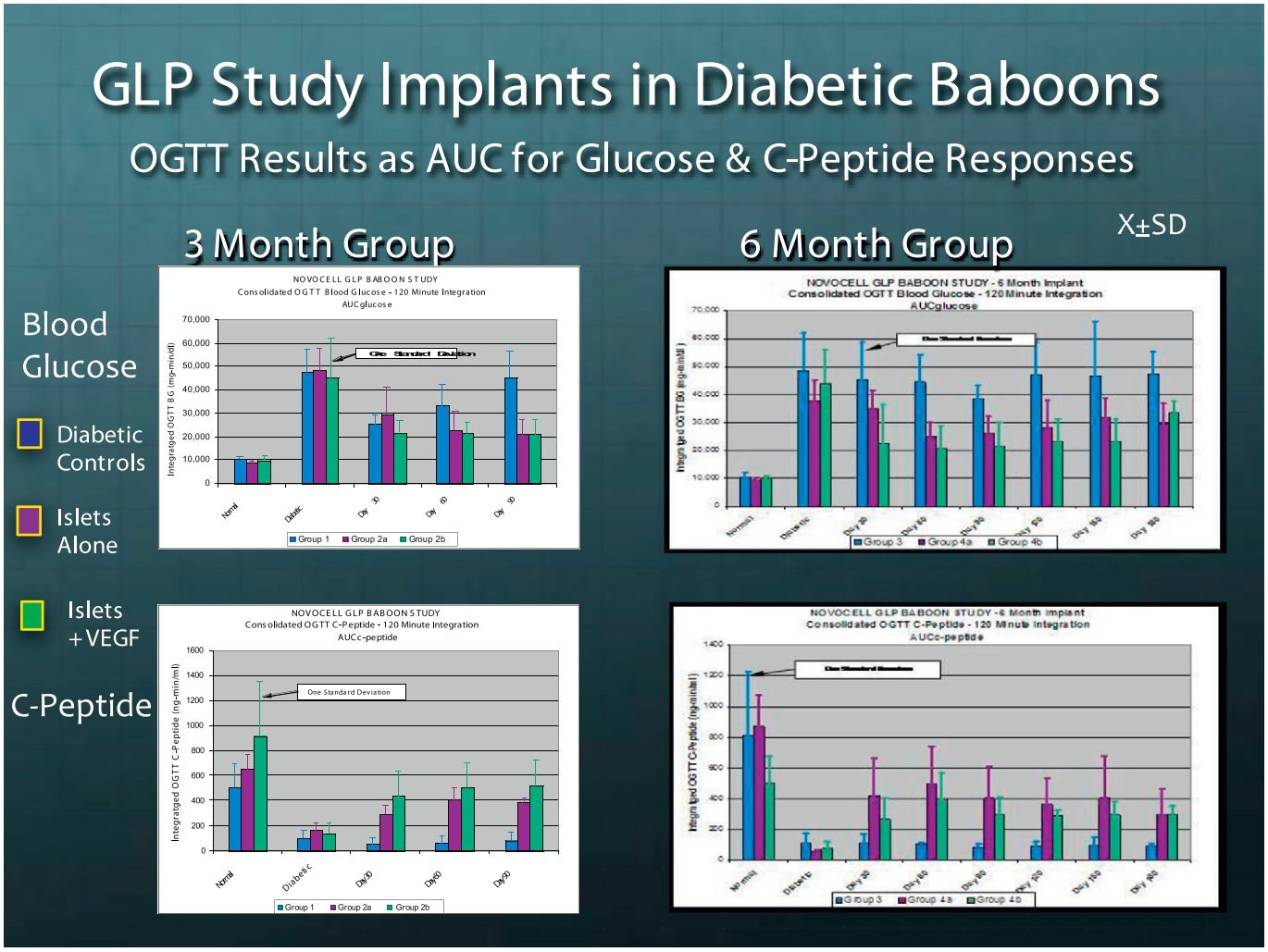

Fig. 3 (*continued*).

strands and lines. Also notice that the encapsulated islets also stain positively for the anti-VEGF receptor. The VEGF receptor staining is not shown for those receiving VEGF at the time of implant as there was significantly more host response in this group.

The anti-inflammatory and anti-immune cell staining in this 3 month group shows very interesting results, again totally different than what was observed in the long-term Pilot study. The permselectivity profiles of these PEG Conformal Coatings on islets demonstrate that the coatings permit diffusion of >400 K Dalton particles, so that IgG at 180 K Dalton should readily cross through the coatings. But, IGM and intact complement should be kept away from the cells by the PEG coatings. The anti-CD68 inflammatory macrophage staining shows a lot of activity outside the capsules in clusters and groups of cells as well as single cells. As shown in the figure below, there is a capsule that no longer shows an islet that is surrounded by layers of these inflammatory cells. Yet, there is no staining within the capsules for this antibody. The anti-HLA-DR staining for dendritic immune cells and activated lymphocytes show clusters of cells outside the capsules and no staining within the capsules. While staining for CD4 positive cells shows both clusters of positively stained cells and linear rows of cells outside the capsules, there is clear evidence of cell staining within the capsules. Knowing the antibodies can readily cross through the capsule, it is not clear as to why they would stain islet cells. Examining the anti-CD8 antibody staining, a different pattern appears in that there is almost no staining outside the capsules of cells. Inside the capsules there is clearly diffuse staining by anti-CD8, but now the staining does not appear to be cellular but rather homogeneous in nature. These findings raise questions that need further studies to resolve.

The histologic results from the 6 month group are similar in some of the cell markers to the 3 month group, but also quite different in other marker expression. The H&E staining is similar to that seen at the 3 month study, as are the insulin and the glucagon staining. The anti-VEGF Receptor staining is markedly less stained in the 6 month versus what was expressed in the 3 month study. But, it is still expressed both outside the capsules and within the capsule with light staining of cells there.

The inflammatory and immune cell special stains show much less activity in the 6 month period after implant than was observed in the 3 month period. There are only a few residual positively staining CD68 inflammatory macrophages remaining with the implants at 6 months. The HLA-DR staining is also essentially gone at 6 months post-implant. The anti-CD 4 staining still shows small groups of positively staining cells throughout the implant site that is far less than what was observed at 3 months as well as positive staining inside some capsules, but with less cell detail. The anti-CD8 cell staining remains scattered outside of the capsules. But now there continues to be diffuse staining within the capsules which appears in cells in some of the capsules. So it remains uncertain as to the actual identity of these immune cells and their intra-capsular versus extra-capsular locations. The histology shown for these baboon studies is for the recipients that

C

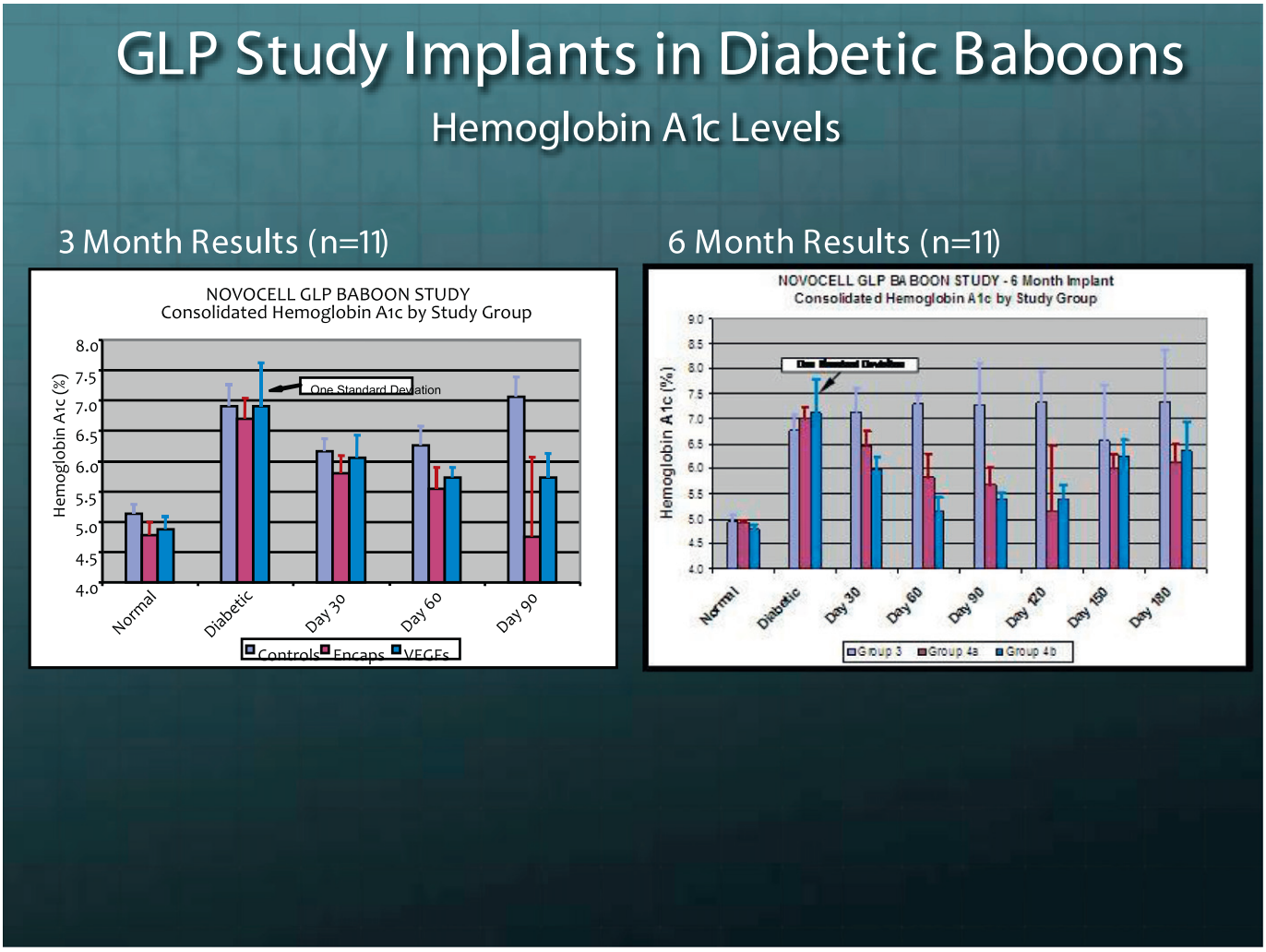

Fig. 3 (*continued*).

received the PEG coated islet allografts that did not have the VEGF analog injected at the time of the implant. Those animals in both the 3 month and the 6 month groups receiving the VEGF analog showed more extensive anti-inflammatory and immune responses as a group at both time frames observed (Scharp not published). Due to this prolonged inflammatory and immune staining, the VEGF analog acute treatment was removed from consideration for the clinical trial.

Summary of the baboon implants in the Pilot and Feasibility Study shows up to 20 months of encapsulated islet allograft function implanted in the subcutaneous site without immunosuppression, except for 30 days of low dose cyclosporine. The results of oral glucose tolerance testing (OGTT) show ongoing function throughout with 50% normal C-peptide responses. The longest recipient required a second implant for slowly reducing graft function on day 420 after the first implant with return to normal blood glucose levels. Histology of the implanted capsules showed well stained islets with minimal inflammatory or immune cell reactions. The definitive GLP study implanted diabetic baboons for 3 months and 6 months with diabetic controls. The responses to the implants were similar to those observed in the Pilot Study with several recipients gaining insulin independence. C-peptide responses were also at the 50% level of normal. Monthly hemoglobin A1c levels confirmed that level of islet function correlated well with human levels of HgbA1c for non-diabetic, pre-diabetic, and diabetic levels. The histology of the implants taken at three months post-implant showed far more reactivity than seen in the Pilot studies at 20 months, not surprisingly so. The histology of the 6 month time showed marked reductions in the acute reactions, to the point of similarity to the Pilot animals 20 month long term histologic results.

2.3.1.2.2. Initial clinical trial of PEG Conformal Coatings. The series of Pilot and Feasibility Studies as well as the GLP Non-Human Primate Studies were sufficiently successful to apply to the FDA for a corporate Phase I/II Clinical Trial of PEG Encapsulated Human Islets Implanted into the Subcutaneous Site in Type 1 Diabetic recipients. The study was approved for 12 patients and the opportunity to enter an accelerated Phase III trial with demonstrated success of the human studies equal to those of the primates with a 6 month primate result lead time required. That study was initiated with preliminary results and funded in part by the Juvenile Diabetes Research Foundation. An abstract of the initial results was accepted and presented as a poster on the preliminary clinical trial results in 2006, but otherwise never published before. Those results are reproduced here [281]. The implant procedure was changed from the baboon trial to include multiple radial injections of 10,000 encapsulated IEQ per injection site as the hub of a wheel with injections from the center outward the length of the needle. The injections were made in subcutaneous sites of the abdomen and the back of the diabetic recipients as separate procedures performed under local anesthesia. The first two recipients were Type 1 Diabetes patients on insulin injections for 25–30 years with fairly thickened subcutaneous tissue due to the long duration of insulin injections. Many of their insulin injections were difficult for them to give, even with rotating their sites. These

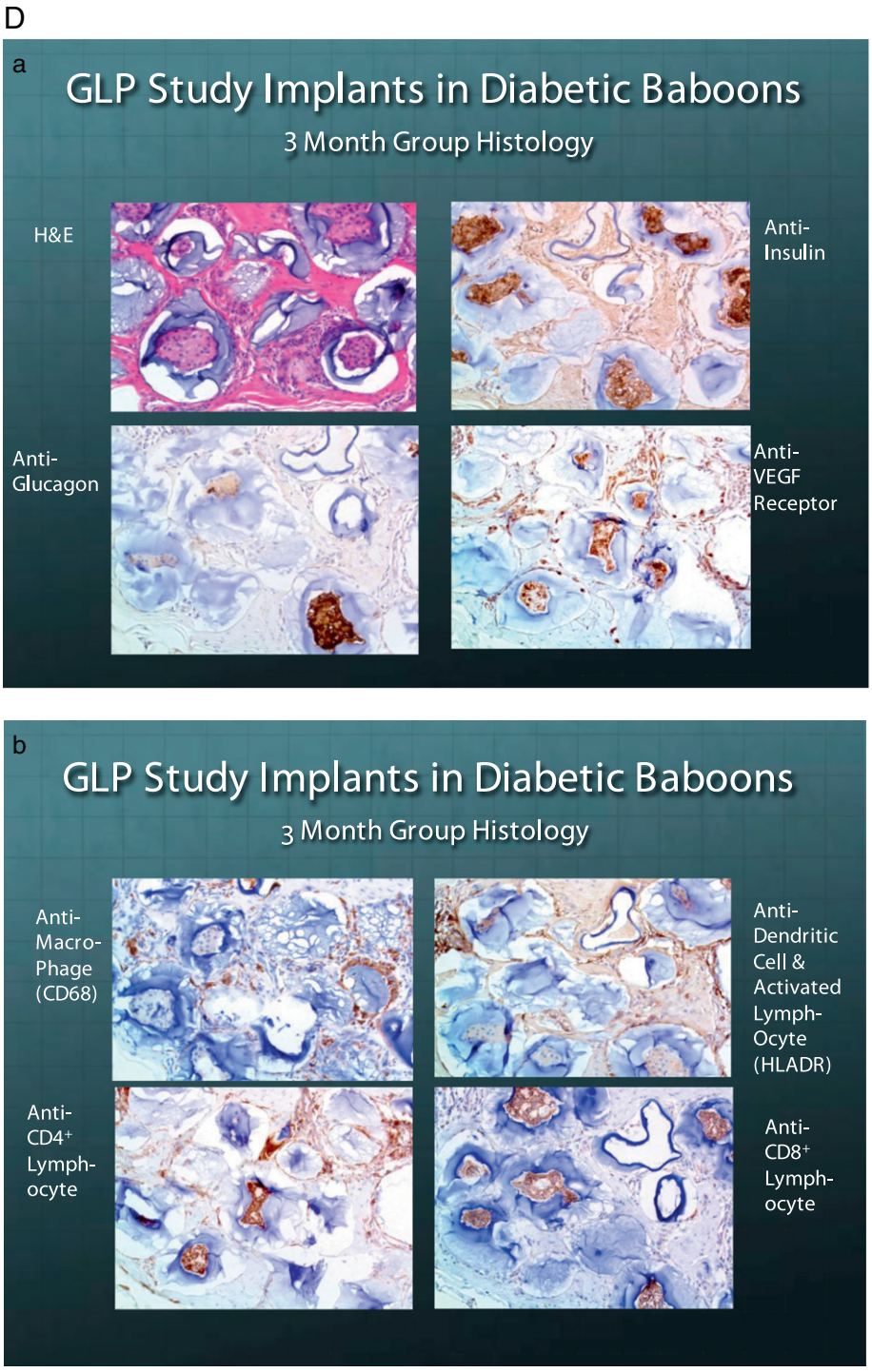

Fig. 3 (*continued*).

human subcutaneous sites were clearly more fibrotic than had been encountered in the short term baboon recipients subcutaneous injections. The Design of the Study is shown below.

Once study candidates were approved regarding inclusion and exclusion criteria and explanation of the trial and its potential risks and benefits, they were enrolled in the study and placed into a potential implant schedule. Pre-implant baseline measurements were taken for 30 days or longer. The subcutaneous implants could be done in several partial implants until the calculated encapsulated islet curative dose was reached. While the FDA had approved 12 donors, the Novocell BOD only approved 2 patients to start the study. There also were only three partial implants performed in each of the two initial patients that were permitted by the BOD to start the clinical trial. The Post-Implant observations were initiated without any observed hypoglycemic or hyperglycemic crises nor ketoacidosis events. As shown in Table 2, the percentage of days that these two recipients spent with both Hyperglycemia (>300 mg/dl) and Hypoglycemia (<70 mg/dl) were recorded prior to the first implants. During the Pre-Implant period, Patient 1 had 54.1% of his days with Hyperglycemia and 43.2% of his days with Hypoglycemia and Patient 2 had similar events with 52.9% of her days with Hyperglycemia and 48.5% of her days with Hypoglycemia. With the three partial implants completed in the two recipients, Patient 1 had received 45%

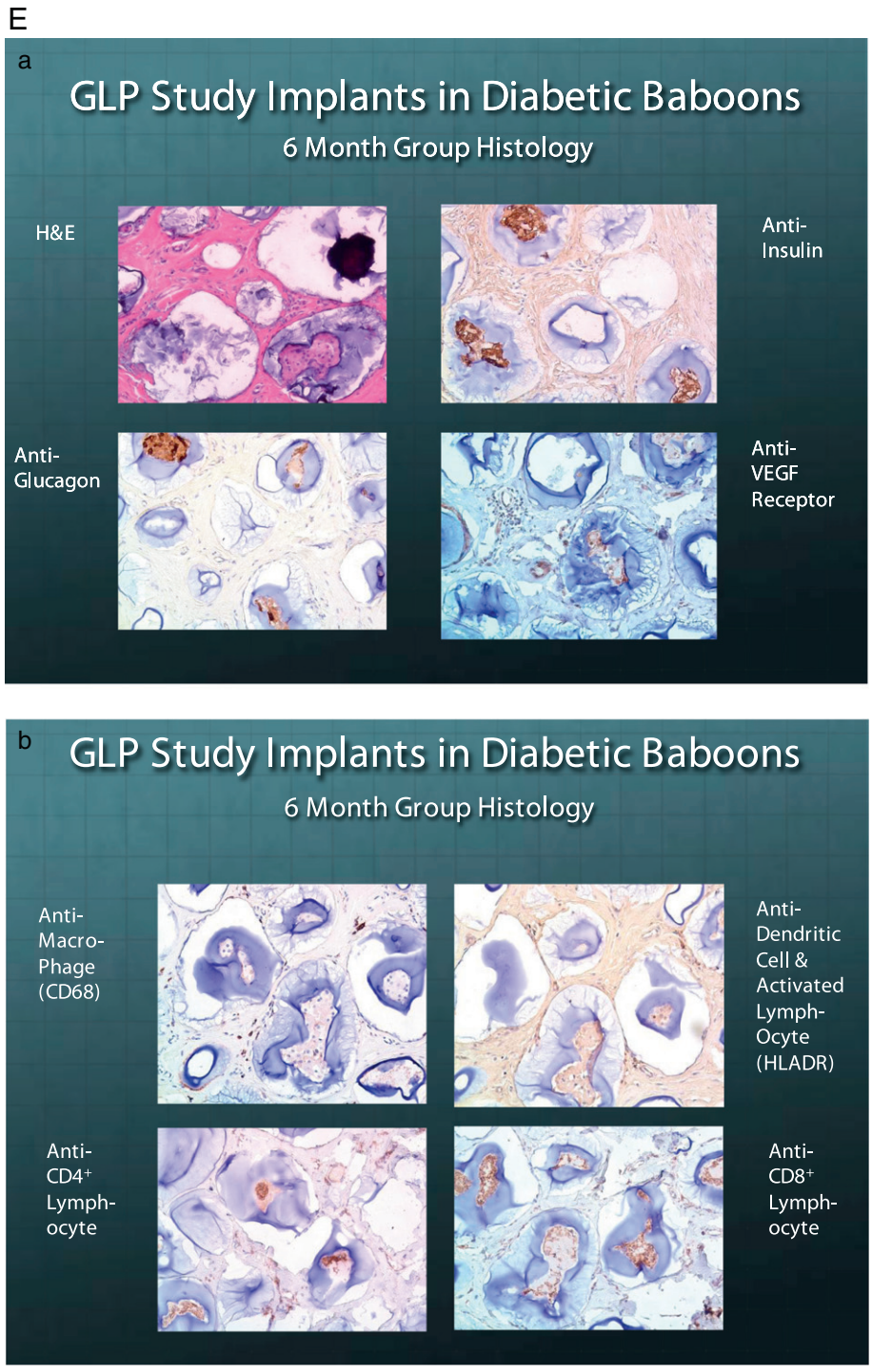

Fig. 3 (*continued*).

of his calculated dose and Patient 2 had received 58% of her calculated dose of encapsulated islets predicted to eliminate insulin requirements. While the percentage of days with extreme blood glucose levels decreased significantly for both recipients after the three partial doses, Patient 1 went from 54.1% to 20.9% Hyperglycemia of his days and Patient 2 went from 52.9% to 33.3% Hyperglycemia of her days. In terms of Hypoglycemic percent of days, Patient 1 percentage of days went from 43.2% to 17.6% Hypoglycemia and Patient 2 percentage of days went from 48.5% to 23.3% of days with Hypoglycemia. These are significant improvements with only partial doses received at this time.

However, neither patient achieved insulin independence during the first 6 and 4 months post-implant, respectively, only receiving an average of 51.5% average of the expected clinical dose predicted to achieve insulin independence. In addition to their significant reductions in both the percentage of days in Hyperglycemia and in Hypoglycemia, both recipients demonstrated decreases in their insulin requirements and significant increases in their OGTT stimulated C-peptide release. Prior to the encapsulated islets implants, neither of the patients had measureable C-peptide levels detected in the blood even with OGTT. After these first partial implants, both recipients had significant levels of C-peptide with Patient 1 at 0.4 pmol/L and Patient 2 at 0.5 pmol/L of C-peptide which are at the level of expecting additional improvements post-implant. While many successful human islet implants under full immunosuppression have higher C-peptide results, there are many with C-peptide starting at

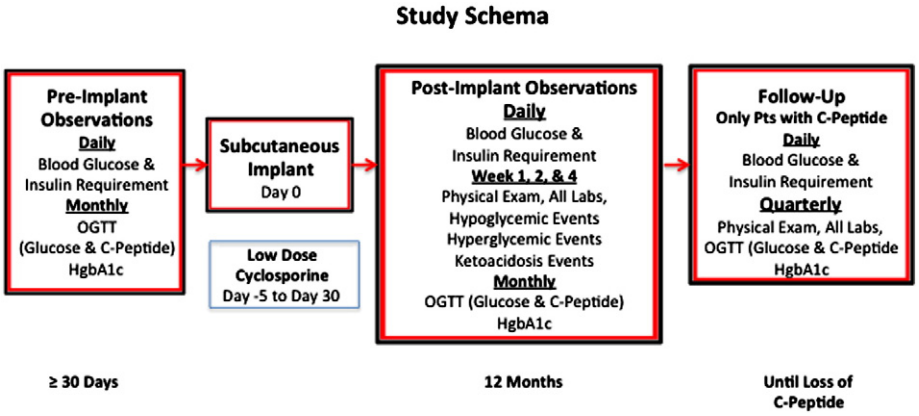

Fig. 4. Design of clinical trial outcomes.

these levels after a full dose of islets [281]. The results in this study are only after a partial encapsulated islet dose. In addition, the Collaborative Islet Transplant Registry minimal requirement for reporting positive islet transplant results is 0.3 pmol/L [282,283]. In terms of islet dose, the clinical trial full dose target was 33,000 IEQ/kg/BW. At 45% of full dose, patient one received 17.5 IEQ/kg/BW and patient two at 58% of full dose received 18.8 IEQ/kg/BW of encapsulated human islets. For reference, the GLP baboon study was completed with an average encapsulated viable islet dose of 47,077 IEQ/kg/BW, demonstrating that only 38% of this baboon dosing was achieved as the clinical dose for these first two patients before the study was closed by the corporate investors.

However, in comparison to the baboon pre-clinical studies, these first two implanted human recipients C-peptide results were lower than anticipated. A significant difference between the baboons and the humans is the subcutaneous implant site in each species, as discussed above. The baboons had only a month of diabetes with their insulin injections and pristine subcutaneous tissues in which to receive the encapsulated islets. Both of the first two human recipients had long histories of subcutaneous insulin injections making it difficult for them to find acceptable sites for their own injections in most areas they could physically reach. Our protocol defined the implant injections at the start of the study. Yet, when performing the implant injections, it became obvious that these fibrous and tough injection sites made the injection of the encapsulated islets more difficult than expected. The most likely explanation of the outcomes being reduced lower than expected is that the insulin released from the encapsulated islets most likely had very slow kinetics in reaching the body. The survival and function of the encapsulated islets after implantation could have readily been less efficient due to the recipients' thickened subcutaneous tissue implant sites. While that is the most likely explanation for the lower level of islet function observed, there is a 2012 publication giving evidence in diabetic mice that barium/alginate encapsulated islets survive well in the peritoneal cavity but not in the unmodified subcutaneous site [284]. Perhaps the subcutaneous site even without scarring has inherent limitations to encapsulated islet implants, especially soon after implant. Back to the clinical trial, at that point in the study, our opinion was that the addition of the final implants would be needed in order for these first 2 recipients to reach their expected outcomes. We also believed it was better to complete these two recipients before moving onto additional patients since their next outcome levels of function should help predict the requirements for the next clinical subjects. Their next implant results should determine if additional patients accepted for the study should be excluded on the basis of potential subcutaneous implant sites with significant fibrosis. Unfortunately, not one of these opinions was able to be tested. In terms of sensitization to these grafts, diabetes antibody samples were run with the first recipient showing no change in these titers, but the second recipient significantly elevated all the diabetes antibodies including pancreatic islet cell, anti-insulin antibody (IAA), GAD antibody, and IA2 antibody. Both had small but increased responses to transplantation antigens. They were not tested for tissue typing for any specific donor antigen sensitization.

2.4. Nanoencapsulation or layer by layer approaches

Nanomedicine relates to tiny measurements at the level of 1–100 nanometers (nm) and it also relates to potential therapies in this scale. In terms of potential applications for diabetes, nano-technical approaches include non-invasive glucose monitoring by implanted nano-sensors as well as nano-encapsulation for islets or a nano-scale implantable insulin pump [285]. Restricting the discussion to nano-encapsulation of islets for the treatment of diabetes, this approach may also be called a "Layer-by-Layer" (LBL) approach as it encapsulates the islets or other types of cell aggregates by altering positively and negatively charged polymers over the surface of the cell aggregate. Primary reasons for developing this approach has been the reality of more standard cell aggregate hydrogel coatings that can leave openings in the coatings, and the diffusional considerations of hydrogel micro-capsules that can decrease encapsulated islet responsiveness to glucose changes. The LBL approach can significantly minimize the capsule thickness, increasing the rate of insulin release in response to glucose (see Formula 1) as well as promote enhanced diffusion of nutrients and waste products. Using this LBL approach, Krol in 2006 coated islet surfaces with polyelectrolytes of polyallylamine hydrochloride and polystyrene sulfonate layers [286]. Similarly, Teramura in 2007 coated LBL layers of polyvinyl alcohol conjugated to a single layer of PEG-phospholipid on the islet surface [287]. In 2008, Wilson described a LBL method of coating

Table 2
First two recipients outcome measurements.

Implant status	Patient #	% Total implant dose	# Partial implants	Months post-implant	% Days hyper-glycemic	% Days hypo-glycemic	OGTT C-peptide (pmol/L)
Pre-implant	1	0	0	0	54.1	43.2	0.0
Post-implant	1	45	3	6	20.9	17.6	0.4
Pre-implant	2	0	0	0	52.9	48.5	0.0
Post-implant	2	58	3	4	33.3	23.3	0.5

poly(lysine)-poly(ethylene glycol)(biotin)(streptavidin) layers on the surface of mouse islets permitting both in vitro and intra-portal vein mouse islet function [288]. In 2010, additional evidence of using poly(ethylene)glycol coating combined with a biotin-PEG peptide conjugates with streptavidin permitted excellent islet coatings. But adding layers of biotin-PEG-GLP-1 increased the insulin secretory capability of the encapsulated islets [289]. More recently in 2012, Pickup demonstrated the LBL encapsulation of islet cells using polylysine and polyglutamic acid coatings demonstrated glucose responsive insulin release in vitro and as well as no adverse effects from the coatings in vivo. They also demonstrated islet allograft success in diabetic mice for a month using this islet coating technique [290]. Also in 2012, Dong demonstrated islet allografts coated with different types of LBL coatings could function for >100 days in diabetic MHC mismatched mouse recipients. They compared uncoated mouse islets with those with pegylated islet surfaces with those with pegylated islet surfaces containing empty nanoparticles and with those with pegylated islet surfaces with nanoparticles containing leukemia inhibitory factor (LIF). There were long term survivors in the last three groups with the longest function observed in those with pegylated islets containing nanoparticles of LIF [291]. The University of Alabama, Department of Chemistry group has recently reviewed these LBL techniques as well as surface modifications of the islets in this rapidly growing new type of islet surface protection. [292]. Another opportunity using nanotechnology is the potential to incorporate minute but locally delivered variety of agents encapsulated within nanocapsules and the islets that can be designed to improve encapsulated islet survival and function.

2.4.1. Layer of cells over surface of islets

Thinking of the difficulties and the variety of approaches to coat islet surfaces completely, the Iwata group has begun a novel approach to layer a coating of living cells over the top of the islets to prevent their immune destruction and aid in the health and function of these cellular encapsulated islets [293]. Their strategy is to take cells from the recipient and place them in a living layer to protect the "cell encapsulated" islets from destruction. To accomplish this, they had to develop a layer that would bind to the islets as well as incorporate the coating cells to act as a living biocompatible layer. To accomplish this feat, they started with a PEG-lipid bound to biotin to create a lipid-PEG-biotin layer onto the islet cell surface. Then streptavidin was immobilized onto the biotin-PEG-lipid bound to the islet cell surface. Since streptavidin has 4 binding sites for biotin, 2 remain open. So the next step was to bind the lipid-PEG-biotin to the coating cells that had been bound with streptavidin as well. These cells are intended to coat the islets which were HEK293 cells for this example. Proof of these layers was accomplished by FTIC confocal microscopy. These coated HEK293 cells had to be able to proliferate in order to coat the entire islet surface. Following the successful coatings, glucose stimulated insulin secretion was demonstrated but at lower levels than normal. Previous attempts at coating islets with cell membranes from chondrocytes had major problems with islet viability [294,295]. Yet, the Iwata group was concerned that since the streptavidin is a xenogenic protein, it might cause immune reactions on the islet surfaces they are wanting to protect when implanted. So in the next publication, they replaced the streptavidin–biotin using a DNA hybridization technique. Taking polyadenine (PolyA) and polythymine (PolyT), they conjugated them with PEG-lipid onto the surfaces of both the islets and the HEK293 coating cells. To accomplish this, they treated the islets with polyA20-PEG-lipid and followed it with PolyT20 with a FITC tag as a second layer so that that the PolyA20 on the islet surface can hybridize with the added PolyT20. With that demonstrated, they then coated the HEK293 cells with the PolyT20 and added them to the PolyA20 coated islets permitting hybridization to bind the two cell types together. But again, it took 3 to 5 days of tissue culture for the bond HEK263 cells to expand and completely coat the islet surface by their expansion. Again glucose stimulated insulin secretion was demonstrated from the coated islets. [296].

In order to achieve a clinically significant islet coating a number of things have to be improved such as finding the right host covering cell type that can be coated to the islets as well as completely cover the islets. Another critical unknown is what the fate of the host coating cells will be upon implantation. Nevertheless, this living cell coating appears to be a unique method to pursue that could completely eliminate all artificial coatings.

3. Host interactions

Following transplantation, the complex interactions between the non-self islet graft and the host involve non-immunological as well as immunological factors, which include innate and adaptive immunity, that can eventually lead to more or less rapid graft loss. Furthermore, since Type 1 Diabetes is an autoimmune disease, destruction of the transplanted beta cells may occur due to the recurrence of autoimmunity. In this section, the potential advantages of islet encapsulation against these mechanisms is discussed, taking into account both in vitro and in vivo studies. However, it must be kept in mind that at least part of these reactions may be elicited by biocompatibility issues related to the coating system materials and conformation, as discussed in a different chapter of this issue. These interactions make it difficult to be definitive as to precise interactions.

3.1. Instant blood-mediated inflammatory reaction

Vascular delivery of islets is associated with early and rapid loss of a significant portion of the graft, that may be as much as 70% of the transplanted islet mass [297]. Coagulation, platelet aggregation, complement activation, neutrophilic granulocyte infiltration and monocyte activation are involved in this aggressive reaction, that is termed instant blood-mediated inflammatory reaction (IBMIR) [298–300]. The extent of the process can be even greater in xenograft models (for example pig islets to non-human primates), due to molecular incompatibilities between the coagulation systems of different species [301]. Several approaches have been tested to prevent or at least attenuate IBMIR, which include pre-treatment of isolated islets, systemic therapies in the recipient and their combinations [299,302,303].

The improved transplantation results obtained with encapsulated islets in several experimental and a few pre-clinical models imply, but do not demonstrate, that encapsulation may protect the islets against IBMIR. However, in the past few years a number of in-vitro and in-vivo studies have more directly addressed this issue using islets shielded by several different approaches. PEGylation, sometimes alone, more frequently in combination with additional modifications, has been the primary model [304–310]. PEGylation involves the addition of linear PEG molecules to the surface of islets as a surface attachment and is not a uniform, encapsulation of conformal coating of PEG as presented above. As discussed [304], Teramura and Iwata modified the surface of hamster islets with a PEG-phospholipid conjugate bearing a biotin group, and this layer was covered by streptavidin and biotin-bovine serum albumin conjugate. The surface was further activated with oxidized dextran, urokinase was anchored to the islets through Schiff base formation, and heparin was added through polyion complex formation between anionic heparin and a cationic protamine. This multilayer approach determined high fibrinolytic activity for approximately seven days, while maintaining in-vitro insulin release, which increased 3-fold upon glucose stimulation. In follow-up studies, the same authors proposed additional approaches to modify islet surface using amphiphilic PEG-conjugated phospholipid or maleimide-PEG-lipid together with bioactive molecules such as urokinase, thrombomodulin, complement receptor 1 and heparin [305–308]. The different islet surface modifications were shown to protect against IBMIR in rodent syngenic [306] and allogenic [308] intraportal islet transplantation as well as in the hamster-to-mouse xenogenic model [305].

A more detailed study was published by Hwang using PEGylated rat islets [309]. Using the in-vitro tubing loop model, the authors showed prevention of platelet adhesion in the PEG treated islets, together with unchanged number of floating free platelets compared to control conditions (blood incubated without islets). When PEGylated rat islets were transplanted into the liver via the portal vein of mice, platelets and neutrophils were rarely found around islet cells, whereas untreated islets were strongly stained with both platelets and neutrophils [309].

A different approach has been reported by Kim who used human endothelial colony-forming cells to coat porcine islets [310]. When the treated islets were examined with the in-vitro tubing loop assay using human blood, platelet consumption inhibition was observed and thrombin–antithrombin (TAT) complex assay results were comparable to those of control experiments. This was accompanied by reduced interleukin-8 levels as well. It may also be possible to utilize PEG-based shielding systems and some degree of immune suppression to protect islet grafts from IBMIR and allograft rejection, as proposed recently in the rat allograft model with the use of an 8-armed-PEG-catechol and N-hydroxysuccinimidyl-linked unfractionated heparin plus the administration of tacrolimus [311]. But this would mean a level of immunosuppression would be required.

In summary, the components of IBMIR may be prevented by several coating approaches, with promising results in islet transplantation studies with small mammals. However, more work is needed to evaluate whether applying the described shielding procedures to micro-encapsulated islets would further protect intravascular islet grafts from IBMIR. There certainly are species differences expected.

3.2. Immune protection

3.2.1. Allografts

Following islet transplantation into the recipient, both the innate (predominant in the first 2–3 days) and the adaptive immune systems respond to destroy the graft [300]. The host immune system is initially activated by the surgical trauma and the introduction of non-self cells. The innate component responds through macrophage and neutrophil activation, causing inflammation in the microenvironment and recruitment of additional immune cells into the graft, initiating a cascade through the release of inflammatory cytokines and reactive oxygen species that damage the islets. Antigens released by the islets are identified and engulfed by antigen-presenting cells (APCs), that process the antigens into the appropriated small peptides that are then presented upon the surface of the APCs in a specific HLA-linked process. The complex is then recognized as foreign that activates helper T cells ($CD4^+$) that in turn activate cytotoxic T cells ($CD8^+$), which eventually destroy the islets [300]. In addition, it has to be kept in mind that the recurrence of diabetes may also lead to selective destruction of insulin producing cells in the graft [312–315]. This concept was first demonstrated with pancreas transplantation [312], when four patients with type 1 diabetes mellitus received segmental pancreatic grafts. The donors were HLA-identical twins in three patients and an HLA-identical sibling in one, and no immunosuppression was given. Despite normal glucose metabolism in the post-transplantation period, impaired insulin secretion function occurred a few weeks later. Pancreatic graft biopsies demonstrated only specific islet infiltration, followed by selective destruction of islet beta cells. This has been confirmed afterwards, with recurrence being associated with changes in autoantibodies [313]. More recently, it has been shown that both allo- and auto-immune processes influence beta cell survival after islet allo-transplantation into type 1 diabetic patients [314,315].

Both macro- and micro-encapsulation have been demonstrated to protect islet allografts in experimental models, with encouraging results obtained in pre-clinical setting and in initial human allograft studies as discussed above and published [82,174,253,316]. Of interest, encapsulation may be useful to also defend the islets against allograft rejection in immunized hosts [317] and to protect from autoimmunity [316,318]. In particular, when diabetic NOD mice received islet allografts under the kidney capsule, they achieved and maintained normoglycemia long-term [319]. After retrieval of islet grafts and return to hyperglycemia, splenocytes were harvested from the NOD mice and transplanted into NOD/SCID mice, that developed diabetes, showing that normoglycemia following micro-encapsulated islet transplantation had been achieved despite maintained islet-specific autoimmunity [319].

Investigations have assessed the potential of encapsulated islet allograft to eliminate human Type 1 Diabetes. In this regard, it is of interest to underline that alginate treatment seems to have only minor effects on human islet transcriptome, as evaluated by microarray experiments [320]. As previously mentioned, a few results have been published on the use of encapsulated human islets for the treatment of Type 1 Diabetes. A case of insulin independence in a Type 1 Diabetic patient was reported after intraperitoneal injection of micro-encapsulated human islets, with micro-capsules made of alginate high in guluronic acid [223]. Afterwards, two phase 1 trials have shown that intraperitoneally infused micro-encapsulated human islets can be considered safe for up to three years after implantation [201,254]. Insulin independence was not achieved in these trials, but glucose control was improved and insulin requirement reduced. Currently, there are four clinical trials retrievable from ClinicalTrials.gov [321] dealing with encapsulated islet allografts (Table 3). Although clearly different in technologies and aims, these studies will hopefully provide useful insights on how to place the use of encapsulated islet allografts in the cure of Type 1 Diabetes.

3.2.2. Xenografts

It is well known that the response of the recipient's immune system to xenografts is generally more aggressive than in allo-transplantation, resulting in rapid and violent rejection due to the presence of preformed antibodies [322]. In addition, xenogenic T cells responses may also preclude long-term acceptance of xenografts [323]. As a matter of fact, pig islets transplantated into non-immunosuppressed, non-human primates are rejected by both humoral and cellular immune reactions [324,325]. In such cases, diffuse and presumably non-specific IgG deposits are observed within islet-associated accumulation of platelets a few hours after transplantation [324,325]. Furthermore, large deposits of IgM and complement are present on islet surfaces 2 to 3 days after xenografting [324–327]. These IgM antibodies are directed against galactosyl and non-galactosyl epitopes, bind to islet surfaces

Table 3
Ongoing encapsulated human islet allograft clinical trials.

Study	Identifier	Status	Sponsor	Comments
Safety and efficacy of PEG-conformal coated islets	NCT00260234	Terminated	Novocell	Subcutaneous implantation in type 1 diabetic recipients
Safety and efficacy of encapsulated human islet allografts	NCT00790257	Recruiting	Cliniques Universitaires Saint-Luc-Université Catholique de Louvain	Alginate-based monolayer cellular device implanted in type 1 diabetic recipients
Beta cell therapy in diabetes type 1 with encapsulated islet allografts	NCT01379729	Recruiting	AZ-VUB	Intraperitoneal islet allograft implants under immune-suppression
Long-term function of beta cell allografts in non-uremic type 1 diabetic patients	NCT00798785	Recruiting by invitation	AZ-VUB	Comparison of intra-muscle, intra-peritoneal, & omental implants of encapsulated islets

soon after transplantation, activate the classical complement pathway and promote neutrophil infiltration [324–328].

Macro- or micro-encapsulation can prolong islet xenograft in several experimental models [65,329,330]. However, several issues remain little investigated. For example, both macro- and micro-capsules may release xenogenic epitopes that can induce the formation of encapsulated tissue specific antibodies [80,196,331,332]. Whether this might or might not be deleterious for the islets is still unclear. Duvivier-Kali micro-encapsulated adult pig islets with alginate and then transplanted the coated islets into the peritoneal cavity of B6AF1 mice made diabetic by streptozotocin injection [196]. The antibody response to the transplanted islets was then assessed by Western blot analysis. In all the studied mice the presence of antibodies against porcine antigens could be detected before transplantation. The presence of newly formed IgG and IgM antibodies was observed in the serum of transplanted mice as early as 5 days after transplantation. Some of the Western blot bands were recognized mainly by IgM and rarely by IgG but other bands were strongly recognized by both IgG and IgM. Therefore, in the model used by Marchetti, encapsulated adult pig islets elicited an early additional immune response, characterized by both IgG and IgM subtypes, that could have been triggered by antigens shed from inside the capsules. Nevertheless, the encapsulated pig islet grafts were efficacious in restoring long-term normoglycemia, suggesting that the coating was able to prevent contact of islet cells with both the host immune cells and antibodies.

The development of a humoral response to micro-encapsulated islet tissue was also demonstrated in a detailed report by Kobayshi et al. [332]. They studied neonatal porcine islets (NPI) micro-encapsulated with alginate and transplanted into the peritoneal cavity of streptozotocin-induced diabetic immune-competent B6 and immune-deficient $B6^{rag-/-}$ mice. Whereas $B6^{rag-/-}$ recipients achieved and maintained normoglycemia for up to 100 days post-transplantation, all B6 recipients of microencapsulated NPI remained diabetic throughout the study. Encapsulated NPI were recovered from B6 mice at day 7 and day 14 post-transplantation were surrounded with a few layers of immune cells that increased with time post-transplantation and contained $CD4^+$ T cells, B cells and macrophages. In addition, anti-pig mouse IgG antibody were measured in serum, that peaked at 30 days post-transplantation, indicating leakage of porcine xenoantigens. Interestingly, by the use of RT-PCR it was found that cytokines (gamma-interferon and interleukin10) and chemokines (monocyte chemotactic protein-1 and macrophage inflammatory protein-1alpha and beta) were expressed in micro-encapsulated NPI recovered from B6 recipients, indicating either local production or that these molecules could traverse the micro-capsule. The study also showed that $B6^{rag-/-}$ mice reconstituted with non-fractionated lymph node cells or $CD4^+$ T cells (but not $CD8^+$ T cells) became diabetic.

However, the composition of the micro-capsules by itself can have a role in affecting absorption of human immunoglobulin on the capsule surface [333]. Tam investigated the adsorption of immunoglobulins (IgG, IgM, and IgA) onto the surface of alginate-poly-L-lysine-alginate microcapsules in-vitro by direct immunofluorescence after their exposure to human serum and peritoneal fluid. They showed that the amount of immunoglobulins adsorbed to the micro-capsule surface was not significantly influenced by the guluronic acid content nor the purity level of the alginate. Importantly, the adsorption was negligible when the poly-L-lysine portion of the membrane was omitted. This suggests that positive charges at the micro-capsule surface are responsible for binding immunoglobulins.

Of more interest is the perspective that pancreatic islet xenografts could be a strategy for the clinical cure of Type 1 Diabetes [334]. In this regard, preclinical studies with encapsulated adult or neonatal pig islets into non-human primates and proof-of-concept studies with coated porcine islet cells into human beings with Type 1 Diabetes are of outmost relevance [82,330,335]. Early encouraging results were obtained with a subcutaneously transplanted macro-device (TheraCyte device, Baxter Healthcare), filled with neonatal pig cells, that was able to maintain cells viable (as shown by insulin and glucagon immunohistochemistry) for up to 8 weeks after xenotransplantation into non-diabetic cynomolgus monkeys [245]. Moreover, after transplantation of porcine islets encapsulated in hollow-fibers with porcine Sertoli cells (which likely have immunomodulating properties) one of twelve non-immuno-suppressed recipient adolescents with Type 1 Diabetes became insulin independent and five children had reduced insulin requirement [336], a result that was received with some skepticism [337]. More recently, correction of streptozotocin-induced diabetes in primates was achieved without immunosuppression by transplantation of pig islets within alginate macro-capsules [338].

As already discussed, studies have been performed with adult or neonatal porcine islets into diabetic Cynomolgus monkeys or human beings [198,257]. In particular, a case report has been published [201] dealing with the long-term viability and function of intra-peritoneally transplanted encapsulated neonatal porcine islets in a diabetic patient. At laparoscopy 9.5 years after transplantation, abundant nodules were seen throughout the peritoneum. Biopsies of the nodules showed opacified capsules containing cell clusters that stained as live cells under fluorescence microscopy. Immunohistology noted sparse insulin and moderate glucagon staining cells. The retrieved capsules produced a small amount of insulin when placed in high glucose concentrations in vitro. An oral glucose tolerance test induced a small rise in serum of immuno-reactive insulin, identified as porcine by reversed phase high pressure liquid chromatography. In 2007, Living Cell Technology launched a phase 1/2a study in Russia of encapsulated neonatal insulin-producing porcine pancreatic islet cells (commercially called DIABECELL®). Some preliminary data have been recently discussed [330]. Seven patients with insulin-dependent diabetes have received between one and three implants of DIABECELL® (5,000 and 10,000 IEQ/kg). Whereas no marked adverse events 18 to 96 wk after transplantation was observed, 5 patients gained normal glucose levels and 2 patients became insulin independent. Following completion of this trial, 2b clinical trials have been launched in New Zealand and Argentina [339]. Hopefully more adequate information on these trials will be given to allow balanced considerations.

However, additional factors should be considered when dealing with islet transplantation, besides immunology and variations in capsule size (macro- vs micro-encapsulation), materials and implantation sites. This is emphasized by the recently reported observation that pigs, non-human primates and human beings have substantial differences in glucose metabolism parameters that could have consequences for transplant outcomes [340].

4. Insulin secretory dynamics from encapsulated islets

4.1. Encapsulated islet function

Pancreatic beta cells produce and secrete insulin to maintain circulating glucose concentrations within a narrow, physiological range. Glucose is the main physiological insulin secretagogue, although several other nutrients, hormones, neurotransmitters and drugs can induce and/or amplify the release of the hormone [341–343]. Normally, glucose rapidly equilibrates across the plasma membrane through specific glucotransporters (glut1 and glut2 in particular) and is phosphorylated by glucokinase, which determines metabolic flux through glycolysis. Reducing equivalents are then produced in the mitochondria by the tricarboxylic acid cycle, that are transferred to the electron transport chain. The energy released is employed to pump protons out of the mitochondrial inner membrane, creating the transmembrane electrochemical gradient. This gradient is used to make ATP from ADP and phosphate. These events result in an enhanced ratio of ATP to ADP in the cytoplasm, which determines the closure of the ATP-sensitive K+ (KATP) channels. This, in turn, results in depolarization of the plasma

membrane, influx of extracellular Ca2 +, and activation of exocytosis of preformed insulin from the granule following fusion with the cell membrane. Classically, glucose-stimulated insulin secretion is characterized by an early phase, which ends within a few minutes, and a more prolonged second phase [344]. In addition, it has been demonstrated that the release of insulin is oscillatory, with relatively stable pulses and variable amplitude [345].

Production and diffusion of insulin (that at physiological pH is a negatively charged protein) from encapsulated islets depend upon several biological and physico-chemical factors [164,171,174,346], many of which are discussed in depth in other chapters of this issue. Whereas research continues looking for optimal conditions [82,171,174,347], in general appropriate selection of capsule components has led to reasonable secretion of insulin in response to glucose stimulation, as assessed by in vitro and in vivo studies. For instance, in macro-encapsulation studies, the use of blending polysulphone with poly vinyl pyrrolidone or sodium dodecyl sulfate was shown not to be suitable for rat islet encapsulation, due to the absence of glucose-induced insulin release [347]. However, hydroxyl methylation of polysulphone allowed efficient insulin release from the macro-encapsulated islets [348]. In early studies from the 80's, permeselective tubular membranes were filled with fragments of human insulinomas, restoring long-term normoglycemia in intra-peritoneally transplanted diabetic rats [348]; after explantation, tissue was studied, showing viable endocrine cells upon histology and electron microscopy examination, and perifusion experiments showing similar results of insulin release for encapsulated and non-encapsulated insulinoma tissue, although variable responsiveness was observed [349].

The release of insulin after retrieval of the macro-encapsulated islets following a period of implantation has been assessed with human islets [111] and rat islets [128], as already discussed in this chapter with the maintenance of beta cell function consistently reported.

Nevertheless, the relative large surface-to-volume ratio of macrocapsules may interfere with optimal diffusion of nutrients and insulin, impairing the regular kinetics of the release of the hormone. For example, rat islets within amphiphilic plymer membranes showed delayed responses to changes of glucose concentrations in vitro (from low to high and from high to low) [350].

In this regard, micro-encapsulation may offer some advantage, and indeed much work has been done with micro-encapsulated islets (of rodent, canine, porcine or human source), which has generally shown in vitro and in vivo insulin release competence of the coated islet cells [82,164,171,174,347,351]. Still, however, the kinetics of insulin release has been reported to be delayed in many studies, which seems to be also related to the volume of the microcapsules [82,164,171,174,347,351,352]. In vitro perifusion work has shown that insulin release from micro-encapsulated islets usually peaks 10–15 min later than from unencapsulated cells, with a similar delay to return toward basal values when glucose concentration is switched from high to low [267]. Similar findings have been reported in in vivo studies. In a comprehensive series of experiments, Tatarkiewicz used alginate to produce rat islet containing micro-capsules [353]. The micro-encapsulated islets showed lower insulin release in response to glucose and glucose plus theophylline stimulation in vitro, when compared to non-encapsulated islets. After transplantation, they cured diabetic mice long-term, but there was delayed delivery of C-peptide from the intra-peritoneally transplanted islet cells. Of interest, when micro-encapsulated islets were retrieved 9 weeks after transplantation, they showed partly maintained glucose responsiveness, with insulin release approaching 50% of that of non-transplantated micro-encapsulated islets [353]. It is possible that the dynamics of insulin release from micro-encapsulated islets may be also affected by the intra-peritoneal location per se. In fact, de Vos found that after gradual infusion of low amounts of insulin into the peritoneal cavity, the rise of insulin in peripheral blood was strongly delayed and reduced in comparison to intraportal insulin infusion [354].

Islet cell aggregates, obtained by dispersing islets into single cells and allowing them to re-aggregate in culture, may offer additional advantages in terms of beta cell survival and insulin secretion function after micro-encapsulation, probably due to better oxygenation [355]. By using this approach with rat islets, improved insulin release in response to 16.8 mmol/l glucose was observed in vitro with micro-encapsulated islet cell aggregates, compared to whole islets, with normal blood glucose levels upon intra-peritoneal glucose tolerance test in grafted mice [355].

As mentioned earlier, isolated islets can be coated by nanomembranes formed by the layer-by-layer technique [174,355,356]. This approach, that can also be automated [357], involves the alternate deposition of polymer electrolytes of positive or negative charge around the islets. The amount of electrolyte deposited in each layer is self-limited, and independent of time and concentration. This conformal protection may conceivably guarantee further improvement as for the kinetics of insulin release, due to absence of dead space around the islets. However, although it has been reported that, mainly depending on electrolyte selection, insulin secretion function from nanocoated islets can be well maintained, studies in this regard are still scanty. In vitro glucose-stimulated insulin release from rat islets protected with alternate layers of phosphorylcholine-derived polysaccharides (chitosan or chondroitin-4-sulfate) and alginate has been reported to be similar to that of uncoated islets [290]. Depending also on the number of layers, nanocoating of human islets with [poly-(allylamine hydrochloride)] (PAH) and [poly-(styrenesulfonate)] (PSS) was associated with preserved secretion of insulin in response to 3.3 and 16.7 mmol/l glucose [286]; however, the use of [poly-(diallydimethylammonium chloride)] was accompanied by blunted insulin release [286]. In another study, PAH and PSS were found to damage human islet cells [288], whereas the formation of PEG-rich coating via layer-by-layer self-assembly of poly(l-lysine)- g-poly(ethylene glycol)(biotin) and streptavidin allowed better insulin secretion function [288]. Therefore, more work is needed to clearly define the insulin secretion function behavior of islets after layer-by-layer nanocoating.

4.2. Long-Term Replacement Strategy

Once islet transplant recipients have received a functional islet graft, their bodies begin to eliminate the effects of diabetes that resulted from the lack of physiological insulin delivery. By the end of 2010, thirty-five thousand diabetic patients in the world had received whole pancreas transplants since 1966 under full immunosuppression with the recent annual numbers of 1200 per year [358]. Recent changes in immunosuppression to avoid steroids following pancreas transplantation have resulted in 95% of the recipients with graft function at 1 year and 83% with graft function at 5 years. Diabetic patients after kidney/islet transplants who achieve the elimination of renal dialysis, diabetes treatment, and hypoglycemic crises clearly report significant enthusiasm for the enormous changes in their quality of life as well as significant reduction of the long-term diabetic complications including their neuropathy, gastroparesis, and loss of limb sensation and pain [359] The most recent clinical islet transplant registry report in 2012 reported 677 islet transplant cases from 1999 through 2010 with the results in the 2007 to 2010 having the most success with 44% maintaining insulin independence at 3 years post-implant. [360] But a more recent report demonstrated that the results of potent induction therapy to islet transplant recipients gave the same 5 year graft function survival rates as whole pancreas transplant recipients given the same induction therapy with both at 50% graft function rate. [361]. Successful clinical islet transplant recipients report similar reductions of their diabetic complications after their transplants function as do pancreas transplant recipients [362]. While many pancreas and islet transplant recipients lose their graft function over time to acute and chronic rejection, many are re-

transplanted, but many of these may not be re-implanted [363]. This results in their return to increasingly severe complications from diabetes that continue to increase returning them to the poor quality of life all patients with diabetes endure from their complications without the benefit of islet transplantation. Adjusting back to these complications is very difficult after having a functional graft for a time.

One of our long term islet transplant recipients under full immunosuppression serves as an example of these changes and the resulting recurrence of these symptoms and complications of diabetes that rapidly returns with loss of graft function. This patient, we will factiously call "Jim", came to our Washington University School of Medicine center in St. Louis, MO, early in our program and received a portal vein islet implant under full immunosuppression as he had received a kidney transplant several years prior [61]. He had suffered long term complications of diabetes including the renal failure, severe peripheral neuropathy and pain, debilitating gastroporesis, retinopathy requiring laser treatments and impotence for many years. His first islet implant only had partial function for a few months so there was no real reduction in his diabetic complications except for transient nerve conduction improvements. A few years later, Jim lost his first renal graft from chronic rejection. Since our center was into a new round of islet transplantation under full immunosuppression, he volunteered again for an islet transplant at the time he received a new kidney transplant. This was successfully accomplished returning him to normal renal function. But this time he received sufficient quantities of islets that he became insulin independent for the first time. Jim's insulin independence lasted for 5 years and continued with partial function for a few more years. One by one, his major complications began to disappear after his return to normoglycemia from the islet and renal grafts. First was the peripheral neuropathy and the pain followed by the elimination of the gastroporesis. His retinopathy stabilized as well. His impotence began to recover first with nocturnal erections and then followed the return of normal sexual function. Being in his forties and having never married due to the chronicity and severity of his diabetes, he married for the first time and began a few years of quality married life. While his second kidney graft was functioning well, he began to slowly lose his islet function and had to return to low dose insulin therapy but only had mild peripheral neuropathy encountered again. Then, Jim was diagnosed with Merkel Cell Carcinoma of the skin around early 2000, having had many years of excess sun exposure working on the family farm in the south. While his Merkel cell cancer diagnosis came prior to discovery of the Merkel cell polyomavirus as the cause of this cancer in 2008 [364], it was clear at that time that the cancer is far more aggressive under immunosuppression. Jim was faced with the decision to stop his immunosuppression and lose both his kidney graft and his islet graft that were functioning and return to his previous state of health with chronic diabetic complications or continue on immunosuppression and hope the cancer would respond to treatment. For Jim, there was only one decision to make, as he could not accept returning to his previous quality of life that he had suffered under diabetes for so many years. So he chose to fight the cancer having lived symptom free for a time without the complications of diabetes. Unfortunately, Jim lost his fight and died from the cancer with functioning grafts.

My purpose in relating this patient experience is that as research investigators, we plan for encapsulated islet therapies to work and function, but recognize the results may only be transient. But when recipients of pancreas or islet transplants see their reversal of diabetic complications and major improvements in their quality of life, understandably, they do not want to hear the word "transient" in explaining that islet transplants do not last beyond 5 years in most recipients. For developing encapsulated islet transplantation without the requirement for immunosuppression, we also have the responsibility to plan for replacement therapy when the implanted islets stop working. As Jim's experience shows us, the impact on the recipients of successful islet grafts changes their lives drastically to the point they do not want to return to the reality of their diabetic complications that brought them to decide to have an implant in the first place. The potential for successful implants now is sufficiently great enough, that we need to be planning for replacement therapy for each patient that is accepted into any encapsulated islet implant program. Thus, our encapsulated islet programs need to provide for replacement grafts from the start. At this time, there are three types of encapsulated islet replacement strategies: a) *All In/Biodegrade Out*, b) *All In/All Out* or c) *Flush/Reload*.

4.2.1. All In/Biodegrade Out replacement

This replacement strategy provides for islet encapsulation components that are biodegradable and will be destroyed by the body's inflammatory processes over time. An example of these is the PEG Conformal Coatings that have esterase sensitive bonds that the body will in time biodegrade [275]. However, that time course in the body at different sites has not been sufficiently investigated to determine very precisely when biological degradation will occur. It appears that it will be far slower than the life of the encapsulated islets from the preliminary results to date. This slow rate of biodegradability for the PEG Conformal Coatings made the decision to not implant them into the portal vein of diabetic recipients, but rather implant them subcutaneously. Alginate or Agarose based islet encapsulation components essentially are not readily biodegraded in the body and will accumulate over time with repetitive implants. There currently is no replacement strategy for these kinds of capsules since they are difficult to retrieve. With the recent development of LBL coatings, this biodegradable replacement approach may be able to be built into the design of these types of islet encapsulation materials. The *All In/Biodegrade Out* is not an approach to consider for macro-devices that induce fibrotic reactions in the host or for the use of human embryonic stem cell derived treatment.

4.2.2. All In/All Out replacement

This replacement strategy can be used on certain types of macro-devices that are designed to not react with the host tissues. These can be hollow fibers containing the islets that are not reactive such as the Cytotherapeutics devices (Section 5) or the Islet Sheet Medical devices made of alginate (Section 5). The Intravascular Devices would also utilize this strategy of replacement. The Beta-O_2 device can utilize this approach for islet replacement since it does not induce severe fibrotic reactions. There could also be certain types of micro-capsules that are non-reactive and theoretically could placed into a site that can be flushed out in order to retrieve them.

4.2.3. Flush/Reload replacement

This replacement strategy can be used predominantly for macro-devices that are designed to grow into the host to provide a vascular interface. The Theracyte device could be an example of this approach in that it clearly is designed to grow into the recipient's tissues. However, the Theracyte device does not have any *Flush/Reload* capability since emptying islets from it simply does not work well as shown by Novocell's inability to convert it (Section 5). To date, no one has developed a successful *Flush/Reload* replacement device for encapsulated islets.

5. History of corporate funding in islet encapsulation

5.1. Descriptions of corporate investigations

The history of corporations involved with islet encapsulation by funding a number of approaches and projects spans over 30 years. The majority of these are start up and small companies. But, several pharmaceutical companies have also been involved in islet encapsulation development. The following Table 4 presents a list of these companies and the type of islet encapsulation technology of interest along with those working with stem cells. The table also shows the pharmaceutical companies that have been involved. Those currently in business and those currently working in Islet Encapsulation are designated. The

Table 4
Types of islet encapsulation technology by company involvement.

Macro-capsules	Sect	Micro-capsules	Sect	Conformal coatings	Sect	Combined	Stem cells
Amicon	5.2.1	Damon Biotech	5.2.7	Neocrin	5.2.8	Biohybrid	Stem Cell Inc[a]
W R Grace[a]	5.2.1	Cell Biotech	5.2.7	Novocell	5.2.8	Neocrin	Cythera
Grace Biomedical	5.2.1	Transcell	5.2.7	Converge[ab]	5.2.8		New Novocell
Circe Medical	5.2.1	Biohybrid	5.2.9				Viacyte[ab]
Cytotherapeutics	5.2.2	VivoRx	5.2.10				Betalogics[ab]
BaxterHealthcare[a]	5.2.3	AmCyte	5.2.10				
Neocrin	5.2.3	TransTech	5.2.11	[c]Beta-Cell			
Theracyte	5.2.3	Encelle	5.2.13				
Islet Sheet Med[ab]	5.2.4	Metabolex[a]	5.2.11				
Cerco Medical	5.2.4	Islet Tech	5.2.11				
Gore Medical[a]	5.2.5	LivingCell Tech[ab]	5.2.12				
Beta 0_2[ab]	5.2.6	Microlslet	5.2.13				
Viacyte[ab]	5.2.3	Islet Sciences[ab]	5.2.13				
Betalogics[ab]	5.2.3	DefyMed[ab]	5.1				
		Encapsulife[ab]	5.2.14				
		Prodo Labs[ab]	5.3				

Sect — refers to the section of the text that follows where each company is discussed.
[a] Designates companies currently in business.
[b] Designates companies currently in islet encapsulation.
[c] Beta-Cell — insufficient information available to include details within this review: www.betacell.org.

Sect title in Table 4 defines the following section number below where each company's islet encapsulation activities are discussed.

The following figure was created to clarify information about the timing of these companies' activities and the duration of interest they maintained on Islet Encapsulation studies. It also shows the start up, changes, mergers and acquisitions, and technology transfers as well as corporate closures. This Fig. 5 defines companies working in Macro-Devices in blue, Micro-Devices in red, and Conformal Coatings in orange. Pharmaceutical companies are shown in green. Those companies working with stem cells are shown in purple. Those companies not working in islet encapsulation are shown in ivory either prior to or after working in islet encapsulation. Those companies that either went out of business or moved out of islet encapsulation are shown with a red X at the time of their closure. Those companies with ongoing business are designated with a horizontal arrow to the right in the 2010–2015 column. The thin black arrows show transfer of technology from one company to another. Following the additional corporate activity graphs, Section 5.2 describes each of the companies or groups of companies' actual activities in Islet Encapsulation. The amount of funding provided by these companies on Islet Encapsulation remains simply conjecture since public disclosure of these details is not usual corporate policy.

As shown in Fig. 6, the peak activity of Islet Encapsulation Companies was from 1990 to 2005 as shown by the total numbers of companies. Similar numbers of companies worked in Macro-Devices and as in Micro-Devices. Two companies worked in both Macro- and Micro-capsules (Table 3). The Conformal Coatings technology started in 1996 and continues onward at a low but steady level. The five-year span from 2000 to 2005 dropped to the lowest numbers of companies at a total of 8. But there is a clear renewed interest for companies coming back into Islet Encapsulation from 2005 to 2010 with the total number back up to 12. The larger pharmaceutical companies came in early in the 1980's but left by 2000. But since 2010, another pharmaceutical company, Janssen Pharmaceuticals, has entered with support of Betalogics.

As shown in Fig. 7 the average duration of companies working from 1980 to 2010 was 8.2 years for those in macro-capsule development and 6.4 years in icro-capsule development with a similar range of 1 to 12 or 13 years respectively. The more recent development of conformal coatings companies are averaging 4.0 years of activity. While the pharmaceutical companies primary activity was early in the history of encapsulated islets, they averaged 8.4 years of activity in the field. The figures calculated for duration have not included the years from 2010 to current.

If the numbers of companies starting up and failing is examined for these same time periods, there are clearly trends as shown in Fig. 8. In the years 1980 to 1985, three Islet Encapsulation companies started up their businesses with one Pharmaceutical company and there were no companies closing their business. After ten years passed in 1990 to 1995, the largest numbers of new companies started up business at a total of 9. But, 6 companies that had started up before failed in 1990 to 1995 and one Pharmaceutical company left the Islet Encapsulation business. The worst time for Islet Encapsulation companies occurred ten years later in 2000 to 2005 with only 2 new companies starting up and the highest total of Islet Encapsulation closing at 6 companies with no Pharmaceutical companies on board. But now, 10 years later, business is looking up in 2010 to 2012 with 6 new companies starting up and only 1 company closing, but also with a new Pharmaceutical company on board. So there clearly appears to be a resurgence of increased interest in the corporate world for support of Islet Encapsulation companies, at least from 2010 to 2012. That should not be surprising with the current terrible trends of increased incidence of diabetes on a global basis trending towards epidemic levels.

There is certainly recent renewed corporate interest and activity in Islet Encapsulation technology since 2010. There is one company working in conformal coatings, Converge with the Hubbell PEG polymer. The four companies working in Macro-Capsules include Viacyte, Betalogics, Islet Sheet Medical, and Beta-O_2. The five current companies working in Micro-Capsules include Living Cell Technologies that has been in clinical trials, Islet Sciences that is a re-start of Micro-Islet, Encapsulife, DefyMed, and Prodo Laboratories. Only two companies of the eleven currently active have been working for five years, Beta-O_2 with an unique oxygen supplying Macro-Capsule and Converge with a new PEG Conformal Coating approach. The others are all newly starting within the last two years to tackle the Islet Encapsulation challenges. Only one has the backing of a large pharmaceutical company, Betalogics from Janssen Pharmaceuticals.

5.2. Summary of corporate activities in islet encapsulation

This section provides brief summaries of all the companies shown in Table 4 and Fig. 5. Listing all of the corporate patents involved is very difficult as they can be assigned to one company and later transferred to another. The patents listed in the report are those listed in the USPTO as being assigned to that company. So there are many additional patents used by these companies that are not easily traceable by this approach.

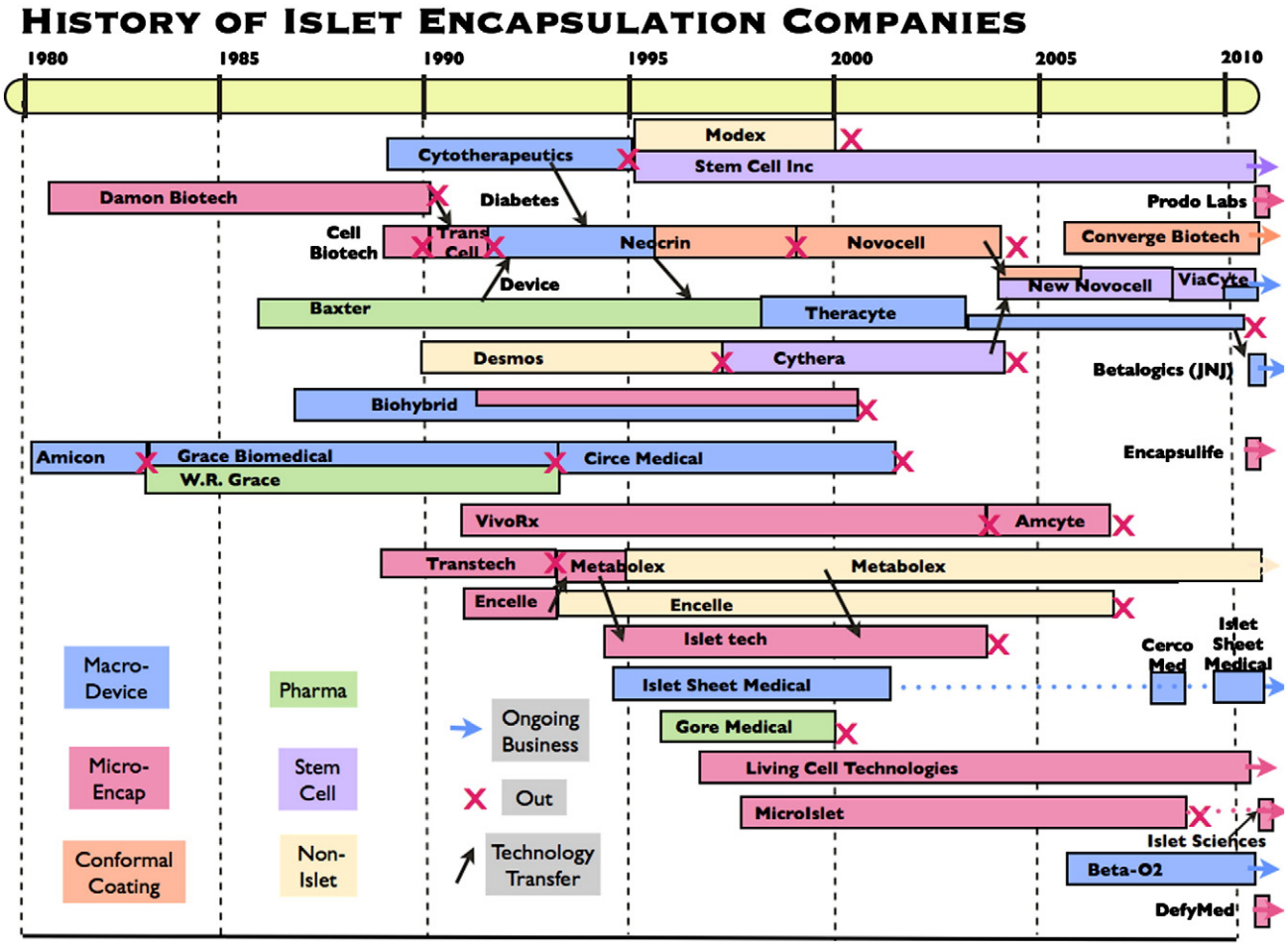

Fig. 5. Corporate Islet Encapsulation efforts.

5.2.1. Hollow Fiber Technology — Group of Amicon, WR Grace, Grace Biomedical, and Circe Biomedical

5.2.1.1. Extravascular devices. All of the initial work was in rodents with the hollow fiber technology that was a difficult product development due to challenges in material biocompatibility and loading a sufficient islet mass. While these efforts were able to get rodents off insulin, they had little success in large animals. This technology was eventually transferred to Biohybrid.

5.2.1.2. Intravascular devices. Product development issues took a great deal of time for Grace Biomedical to develop a functional intravascular device encapsulating islets for large animals. But their efforts were successful in terms of tolerance of the recipient to an in vivo intravascular device without thrombosis in dogs. They were also able to demonstrate the ability to load a sufficient islet mass in their "hockey puck" device for ongoing islet function for several months. In review, their data generated in large animals stands as the most successful and longest to date for xenograft islets (porcine & bovine) into diabetic dogs. They came very close to an FDA approval to actually start a clinical trial. But, unfortunately, the last few dogs with long term functional islet grafts suddenly died within a short time of each other when their vascular connections failed resulting in extensive acute blood loss. This catastrophic vascular interface failure resulted in the permanent closure of

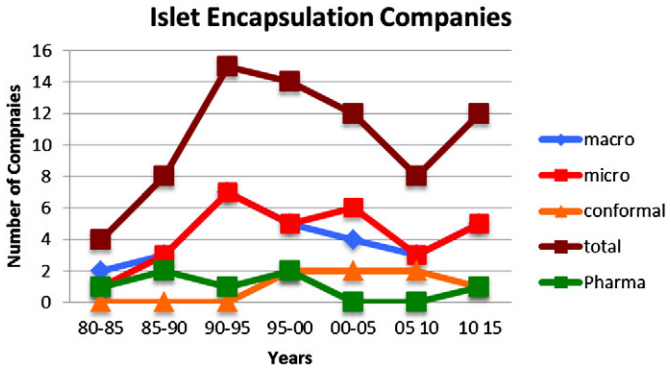

Fig. 6. The islet isolation company types of encapsulation technology.

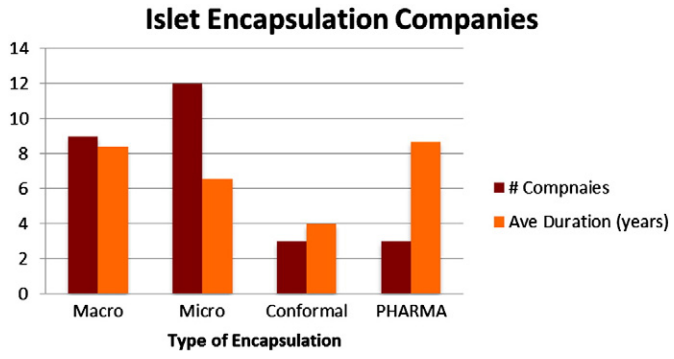

Fig. 7. Average duration of companies working in encapsulated islets.

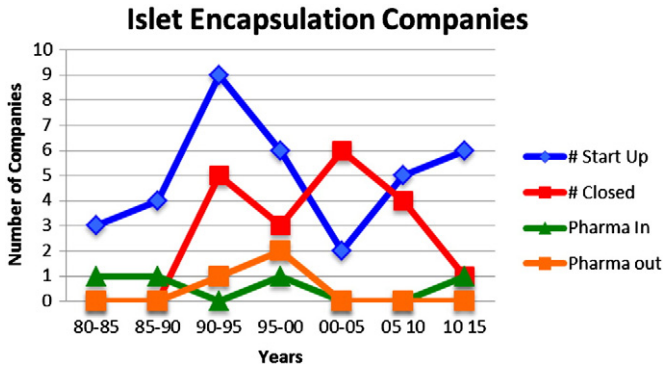

Fig. 8. Trends in islet encapsulation companies start ups and closings.

this approach for diabetes. Yet, Cerce Biomedical took the device for a rescue of acute liver failure product as an external vascular device where risk of vascular failure could be monitored. However, since they were never able to maintain sufficient viability of their encapsulated hepatocytes to gather meaningful clinical data in a Phase 2 trial, the company was closed and the technology development stopped. The following patents were issued to cover this technology: W.R. Grace was issued 3 USPTO patents to protect their technology: [365–367].

5.2.2. Hollow Fiber Technology — Cytotherapeutics

5.2.2.1. Extravascular devices. Similar hollow fiber technology as developed by the previous group were used in rodents with similar success by Cytotherapeutics. Implants into larger animals resulted in partial islet function. A non-curative Investigator IND approved by the FDA in 1993 implanted human islets into a subcutaneous site in Type 1, Type 2, and non-diabetic humans in a non-curative study. Results demonstrated successful protection from both autoimmune recurrence and allograft rejection with functional islets recovered in all groups. However, packing density survival was at 5% that would not translate well into a commercial product due to excessive lengths of fiber required to cure diabetes in humans. This technology for diabetes was transferred to Neocrin but not developed further. Cytotherapeutics continued its cell encapsulation technology into its parallel neurologic program, but was unable to develop a functional device for Parkinsonism due to its inability to develop a functional tumor cell source that had the required FDA historic rigor for cells lines and adequate function. They turned to a clinical product for the treatment of chronic pain using encapsulated bovine adrenal medulla cells. But, clinical trial outcomes for chronic pain patients failed to meet required endpoints losing the opportunity for approval (Fig. 4). They sold the cell encapsulation technology to a cancer company in Europe where it was never further developed. The biological side of Cytotherapeutics was then merged into Stem Cell, Inc. that remains a viable company in San Francisco, but not working in Diabetes. The following patents were issued to cover this technology: Cytotheraputics was issued 20 USPTO patents and Neocrin was issued 3 USPTO patents to protect their technology: Cytotherapeutics [368–387], Neocrin [388–390].

5.2.3. Supported flat sheet device — Group of Baxter Health Care, Neocrin, Theracyte, Viacyte, and Betalogics

5.2.3.1. Extravascular device. Baxter Health Care had designed and built this device for delivery of cell products to be delivered from its genetic engineering division. Yet, their gene therapy program was unable to develop a successful gene product to be encapsulated in the device. So they turned to Diabetes as a model during that development and formed a joint venture with TransCell to form Neocrin to develop the device for diabetes. While successful in rodents as an allograft device with open porosity, they claimed the rodent success was due to stimulation of vascularity around the diffusive membrane. But this vascular induction was not able to be confirmed in larger animals with curative results. Neocrin's collaboration with Baxter was able to convert the alloprotective membrane to one that would permit survival of xenograft cells. But their large animal testing was not able to translate the rodent membrane vascularization success. One of the major challenges for this type of device is to be able to restore encapsulated islet function when the old islet batch needs to replaced with new islet loads. Since this device stimulated host overgrowth by design, it was not a device candidate for “all in/all out” implants that prevented it from being removed and replaced easily. Any device encapsulating islets has to have the ability to replace them when the islet function is lost. Neocrin tried to convert this macro-device to a “flush/reload” device to replenish the islet load since the host tissue grew into the outside layers of the device. Neocrin was not able to resolve this challenge and returned the device to Baxter in order to develop Neocrin's PEG conformal technology that was being developed in parallel. Baxter then spun the technology out to Theracyte that sold the devices to academic and corporate entities for research and development for several years. With this encapsulation technology going off patent, two companies are currently revising it for their use for encapsulating human embryonic stemm cell derived insulin-producing cells. One of these is Viacyte. The other is Betalogics, a division of Janssen Pharmaceuticals. Baxter Health Care was issued 25 USPTO patents and Viacyte was issued 8 USPTO patents: Baxter Health Care [391–415], Viacyte [416–423].

5.2.4. Minimally supported extravascular device — Islet Sheet Medical & Cerco Medical

5.2.4.1. Extravascular device. Islet Sheet Medical initially developed this device as one made of minimally supported flat sheet, permselective membranes that have tubing attached for loading. Rodent results showed success, but difficulties demonstrating viable large animal results were encountered due to disruptions and foldings of flat sheets in vivo in the more challenging large animal model. The company closed for a time, but was re-financed as Cerco Medical that continued development, but then closed. This effort and approach was recently refinanced as Islet Sheet Medical and is now working in large animals to develop a clinically relevant device for Diabetes. Islet Sheet Medical was issued 3 USPTO patents to cover their technology: Islet Sheet Medical [424–426].

5.2.5. Uncertain type of extravascular device — Gore Medical

5.2.5.1. Extravascular device. Gore Medical was supplying the Baxter/Theracyte device the membrane within the Baxter device that stimulates vascularization in the rodent host. With the closure of the Baxter encapsulation effort, some personnel left Baxter for Gore Medical to develop their own Macro-device. But, after a time when there were no results presented publically, the effort was closed within the company without technology disclosures. Even with this closure without public results, Gore Hybrid Technologies was issued 2 USPTO patents to cover their technology: Gore Hybrid Technologies [427,428].

5.2.6. Mechanical extravascular device supplying oxygen to islets — Beta-O_2

5.2.6.1. Extravascular device. This company is currently developing different ways of supplying oxygen to encapsulated islets in a “pace maker” type of implant. The device has an islet compartment and different types of methods to supply oxygen to the graft while implanted, including direct influx of external oxygen to the islet compartment in the device separated from the oxygen flow by a semipermeable membrane. With successful rodent data recently reported, their current effort is in developing the technology for

implants into large animals with diabetes and eventual clinical trials. Beta-O_2 was issued 1 USPTO patent to cover this technology: Beta-O_2 [429].

5.2.7. Alginate micro-capsules — Connaught Laboratories and Damon Biotech

5.2.7.1. Extravascular device. These were two early companies developing Tony Sun's alginate micro-capsules towards clinical implants. Sun's first reports showed long term success in rodents that was the pivotal publication that began most of the alginate microcapsule effort that has followed and continues. This technology was licensed to Connaught Laboratories. There has been difficulty repeating these rodent results by others, but long after the closure of Damon Biotech, Sun reported excellent long term success in diabetic Non-Human Primates recipients. Damon Biotech had major trouble in translating the technology to large animals from several different aspects. They offered the technology out to other companies resulting in the technology to be sold to TransCell. But no progress was reported and it was not followed up into products. TransCell was converted into Neocrin when the Baxter device became available. Connaught Labs was issued 1 USPTO patent and Damon Biotech was issued 1 USPTO patent to cover this technology: Connaught Labs [430] and Damon Biotech [431].

5.2.8. Alginate micro-capsules to macro-devices to conformal coatings — Group of Cell Biotech, TransCell, Neocrin, Novocell, and Converge

5.2.8.1. Alginate micro-capsules. This technology that had been transferred from Cell Biotech to TransCell was not followed up as a separate technology. Instead, it supported the matrix of the Macro-Device that did follow with islets.

5.2.8.2. Macro-capsules. These efforts were discussed in the previous Baxter Healthcare group section in 6.2.3.

5.2.8.3. PEG Conformal Coatings. This Jeff Hubbel technology was jointly developed by Neocrin and Novocell and licensed to both companies. N. Desai was a post-doctural fellow with Hubbell at UT during the development and left to join P. Shoon-Shiong at VivoRx (5.2.10.1) who filed their own patent on this work that was approved. After more than 10 years of legal work, the USPTO decided that the patent rights belonged to Neocrin. These rights were transferred to Novocell. Neocrin took it successfully through the rodent phase but was not able to complete the development in large animal models due to their reactivity to the components and the porcine xenograft islets. Novocell was started with human islets as the source, eliminating the xenograft problem. Rodent studies were very successful. A large number of Non-Human Primates were implanted successfully with encapsulated islet allograft implants in the subcutaneous site with elimination of insulin requirement for 20 months without long term immunosuppression. A clinical trial was approved and partially funded by the Juvenile Diabetes Research Foundation (JDRF), but stopped after two patients since insulin independence was not achieved. Novocell had merged with Cythera in 2004 forming the New Novocell that combined the human embryonic stem cell approach to newly created insulin-producing cells with the conformal coating Islet Encapsulation. But, Novocell closed down the Irvine conformal coating facility in 2006 and abandoned the encapsulation technology to focus on their human embryonic stem cells to islet cells efforts and returned to San Diego. Paul Latta, former CEO of Novocell formed a new company, Converge, working with a second generation approach from Jeff Hubbell of the PEG Conformal Coating. They are working with the Diabetes Research Institute in Miami and have passed successful rodent studies. They are currently working on non-human primate studies with their conformally coated islets. Cythera was issued 8 USPTO patents and Novocell was issued 5 USPTO patents to cover their respective technology: Cythera [432–438] and Novocell [439–443].

5.2.9. Hollow fibers and alginate micro-capsules — Biohybrid

5.2.9.1. Hollow fibers. Bill Chick was involved in the Amicon and the WR Grace development in the use of hollow fibers in the extravascular sites. He then founded Biohybrid prior to the intravascular product to focus on this technology to study extravascular device development. Rodent work was successful, but large animal applications proved difficult.

5.2.9.2. Alginate micro-capsules. Biohybrid also spent a great deal of energy to develop alginate Micro-Capsules into a clinically relevant product, but was not successful in achieving consistent large animal results. After many years of development, this company closed following Chick's death due to complications of Type 1 diabetes. Biohybrid was issued 6 USPTO patents to cover this technology: Biohybrid [444–449].

5.2.10. Alginate & PEG micro-capsules — Group of VivoRx and Amcyte

5.2.10.1. Alginate/PEG based micro-capsules. Patrick Shoon-Shung started CellBiotech but left TransCell to start VivoRx using similar alginate to alginate/PEG to PEG Micro-capsules. There were a number of large animal and human implant results published using the VivoRx technology that have not been reproduced by others. After several years, VivoRx closed and reopened Amycte to continue the work. But, Amycte closed without developing significant large animal results using this technology that was not transferred at the time of the second closure. VivoRx was issued 14 USPTO patents and Amcyte was issued 2 USPTO patents to cover this encapsulation technology: VivoRx [450–463] and Amcyte [464,465].

5.2.11. Alginate micro-capsules — Group of TransTech, Metabolex, and Islet Tech

5.2.11.1. Alginate micro-capsules. TransTech Started with alginate microcapsules by licensing patents from the University of California San Francisco but transferred this technology to Metabolex. They developed small animal results but their large animal results were difficult for them to duplicate. Metabolex turned away from islet encapsulation to focus on pursuing small drug discovery and sold their islet technology to Islet Tech. It pursued alginate purification technology and alginate small and large animals results, but were not consistent in developing pre-clinically relevant results in the large animals and closed. Trans Tech licensed 4 patents from the Reagents of the University of California and was issued 2 USPTO patents and Metabolex was issued 6 USPTO patents to cover this technology: Trans Tech [466–469] and Metabolex [470–475].

5.2.12. Alginate micro-capsules — Living Cell Technologies

5.2.12.1. Alginate micro-capsules. LCT began with efforts to combine islets with Sertoli cell implants but was unable to stabilize the commercial culture of stable Sertoli cells and turned to alginate encapsulation of islets. Their technology using porcine islets into rodents and large animals progressed to large animal results that supported the approval of clinical trials. Two early, small clinical trials were performed with one in Moscow. A second trial was approved in New Zealand and is currently under way. Only preliminary results have been disclosed at this time that appear to be promising. LCT was not issued any USPTO patents to cover this technology but has licensed and issued patents in other countries.

5.2.13. Alginate micro-capsules — Micro-islet

5.2.13.1. Alginate micro-capsules. Micro-Islet began several years ago developing alginate based islet encapsulation technology. Along the

way, Encelle's "stealth" technology, originating at Duke University was licensed and incorporated. Rodent data was completed and large animal implants were performed in Non-Human Primates using encapsulated porcine xenograft islets, but these results remain within the company. Work was started to enter a Phase I clinical trial, but the company went bankrupt and closed prior to achieving these goals at the end of 2008. A new company, Islet Sciences, has recently been formed and funded by similar investors and scientists and is initiating research and development of Micro-Capsules. Encelle was issued 7 USPTO patents to cover this technology: Encelle [476–482].

5.2.14. Hydrogel micro-capsule patch — Encapsulife

5.2.14.1. Hydrogel microcapsule patch. Taylor Wang, PhD has worked several years on developing a centralizing hydrogel combination micro-capsule for islet encapsulation and published successful rodent and dog implant studies. He recently formed Encapsulife to continue the development of this approach in large animals towards a clinical product. Encapsulife has not been issued any USPTO patents to date to cover this technology: Encapsulife (none).

5.2.15. Additional Patents that were licensed to different companies

These were assigned to the companies listed, but have been sold to various companies.

Circe Biomedical [483–485], Damon Corporation [486], McGill University [487,488], and Zimmerman patent not assigned [489].

6. Summary of progress

There has been significant progress made in the last several years with islet encapsulation. In the Macro-Device approach, there are two approaches in the Extravascular Diffusion Device section in large animal studies currently (Islet Sheet Medical and Betalogics) with the Betalogic oxygen supplying device anticipated to be in clinical trials within a couple of years. In this same group of Extravascular Diffusion Devices, there are two companies trying to make the jump from diabetic mice to direct human implants with very similar Theracyte-like devices, Viacyte and Betalogics. Both of these companies are developing human embryonic stem cell differentiated human islet products that require robust devices to protect from any potential of undifferentiated embryonic cells escaping the devices. Without a large animal embryonic stem cell differentiated islet model available, both companies require the FDA to approve a mouse to human jump to gain approval for clinical trials. Both appear to be near application to the FDA to initiate these clinical trials in the next few years. In spite of excellent long term encapsulated xenograft results several years ago in the Intravascular Diffusion Devices, no one is currently taking up the challenge due to the artery-device interface challenge that stopped this type of device in the early 1990's.

In terms of the Micro-Devices, the alginate-barium microcapsules for islets are moving forward with a much smaller capsule size that has clinical relevance. In fact, Living Cell Technologies is currently in their third clinical trial using encapsulated pig islets or neonatal pig islets with the earlier two trials showing marginal promise in terms of long term graft function. There has been a number of pre-clinical studies showing promise with increasing survival of islet mass and exploring non-standard immunosuppression approaches for xenografts. While there have been several pig, dog, and non-human encapsulated islets with excellent long-term results, none of these centers seem ready to initiate clinical trials. The Wang Multi-Component Alginate device posted excellent dog results in the last few years so they could be considering a clinical trial as well. Otherwise, there have been recent advances in Alginate Emulsification Coatings that could readily advance to large animals in the near future.

This publication was able to present pre-clinical as well as preliminary clinical trial results of the Hubbell PEG Conformal Coatings for islets at Novocell that have been held from publication for several years. Up to 20 months of insulin independence without immunosuppression in non-human primates was demonstrated in the pilot studies with additional positive results from the Good Laboratory Practices (GLP) studies needed for clinical trial application. While the Novocell Phase I/II clinical trial was FDA approved, it was stopped after two partial implants since the patients did not achieve insulin independence. Hubbell is currently working with Converge developing the next generation of PEG Conformal Coatings with positive large animal results in progress that could lead to another clinical trial in the near future.

The latest in islet encapsulation of Layer-By-Layer coatings clearly show promise in their preliminary studies. In fact, the first cell coatings used to cover islets are also beginning their development. The first demonstration of successful vitrification of agarose encapsulated islets eliminating rodent diabetes may prove an important way to store quantities of islets for clinical application. While the 5 primary concerns remain in the way of a clinical therapy, there is clearly progress in the first four: a) poor translation of rodent results to humans, b) hypoxic losses of islets post-implant, c) elimination of residual immunosuppression, and d) insufficient quantities of human islets for transplantation through the human embryonic islet programs. But little progress has been made on the risk of sensitizing islet transplant recipient volunteers for experimental studies. The additional critical needs for encapsulated islet clinical therapy are focused in Table 4 for consideration that follows.

7. Critical needs required for clinical success

As reviewed in this article, there has been a great deal of research and development done by both university and corporate investigators to bring islet encapsulation technology to the clinic (Table 5). Below is a summary of the collected thoughts of the co-authors and contributors regarding the critical needs remaining to deliver a clinical therapy.

8. Addendum

Following the initial publication of this article, VIACYTE requested the following addendum be added which has not been previously published, meaning it has not been peer-reviewed nor verified by the author.

> "While 12 patients were approved by the FDA, 5 patients were enrolled and 2 patients were treated to almost the full dose of 60,000 IEQ/kg with the first patient receiving 58,076 IEQ/kg (97%) and the second patient receiving 54,684 IEQ/kg (91%). Due to these nearly complete doses implanted into these two patients with Type 1 Diabetes without demonstrating insulin independence, Novocell stopped the clinical trial and closed the study."
>
> [A. Robins, personal communication, Viacyte, 2013]

Clinical graft functional results were not included in this follow up. For reference, one can compare their results added here to those after partial implants that were published in 2006 at the American Diabetes Association (281) and summarized in Table 2 in Section 2.3.1.2.2 of this publication. As provided in Table 3 in Section 3.2.1 of this publication, the Novocell Clinical Trial information is available at www.clinicaltrials.gov under reference #NCT00260234, and confirms trial termination without any clinical data entered with their last verified change in August, 2006.

Acknowledgments

Investigators participating in the Novocell Phase I/II Clinical Trial

The co-authors acknowledge the participation of the following investigators in the design, patient recruitment, and performance of the Novocell Phase I/II Clinical Trial: Sherwyn Schwartz, MD, Diabetes and Glandular Disease Clinic, San Antonio, TX, and Paraic Mulgrew, MD, Renal Associates PA, San Antonio, TX. Jose Oberholzer, MD,

Table 5
Critical needs required for clinical therapy.

Critical need for islet encapsulation	Choices	Primary vs secondary requirement	Large animal results	Human clinical therapy
Biocompatibility	No reactivity to membrane, capsule, or coating	Primary	Required	Required
Unlimited islet source		Primary		Required
	Expanded human islets		Xenograft	Acceptable
	Porcine islets		Acceptable	Acceptable
	Matured fetal stem cell derived human islets		Xenograft	Acceptable
	Maturity required in vivo for fetal stem cell islets		Xenograft	Acceptable
	Adult stem cell derived human islets		Xenograft	Acceptable
Immune protection		Primary		Required
	Auto-immune TID		NA	Required
	Allograft immune		Required	Required
	Xenograft immune		Required	Required
	Immunosuppression		Not acceptable	Not acceptable
Islet oxygenation		Primary		Required
	Pre-vascularization		Acceptable	Acceptable
	Oxygen supplied		Acceptable	Acceptable
	Oxygen carrier within		Acceptable	Acceptable
	Arterial connection		Acceptable	Acceptable
Functional insulin release kinetics	Prevent significant hyper- & hypo-glycemic excursions	Primary	Required	Required
Functional implant site		Secondary		Required
	Subcutaneous		Acceptable	Acceptable
	Intra-peritoneal		Not NHP	Doubtful
	Omental		Acceptable	Acceptable
	Hepatic		Risky	Risky
Device/capsule islet replacement		Secondary		Required
	All In/Biodegrade Out		Acceptable	Acceptable
	All In/All Out		Acceptable	Acceptable
	Flush & Reload		Acceptable	Acceptable
Donor antigen sensitization		Secondary		Protection required
	Xenograft islets		Acceptable	Acceptable
	Allografts — common donor antigens		NA	Risky for organ Tx
	Allografts — uncommon donor antigens		NA	Acceptable

University of Illinois in Chicago participated by supplying human islets as part of these encapsulated for the clinical trial.

Paul Latta and Jeff Hubbell for PEG Conformal Coating

The co-authors acknowledge the skill and efforts of Paul Latta, co-founder of Novocell, in building that company and the PEG Conformal Coating Islet Encapsulation product presented within this review. He also needs to be credited for founding Converge and the product development work he is doing with Jeff Hubbell on the next generation of PEG Conformal Coating for Islet Encapsulation. Jeff Hubbell is the original inventor of the PEG Conformal Coating along with so many of his accomplishments and is acknowledged for continuing this product development for diabetes therapy.

Juvenile Diabetes Research Foundation in Novocell Phase I/II Clinical Trial

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Investigators contributing their key literature for review & their lists of remaining critical needs

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Contribution to history of islet encapsulation companies

The co-authors acknowledge the contribution of this investigator's collected information regarding participating companies in encapsulated islet development: Scott King, Islet Sheet Medical, San Francisco, CA.

Appendix A. David W. Scharp, MD — Islet Encapsulation Corporate Experience

David Scharp began his corporate activities in Islet Encapsulation in 1989 in the formation of Cytotherapeutics funded by Mayfield VC. While located at Washington University School of Medicine in St. Louis, MO, Paul Lacy, MD, PhD and David Scharp, MD were founding scientists for the diabetes encapsulation products for the company. Pierre Galletti, PhD and Patrick Aebischer, PhD from Brown University were founding scientists for the neurologic cell encapsulation products. Thomas Chang, PhD from Magill University was a founding scientist for micro-capsules. Scharp and Lacy performed contractural research for the company in diabetes including an FDA approved Investigator New Drug clinical trial of the hollow fiber Islet Encapsulation technology. The cryopreserved and encapsulated human islets from a single donor were implanted in the subcutaneous site into 9 human subjects: 3 non-diabetics, 3 with Type 1 Diabetes, and 3 with Type 2 Diabetes in 1992. This non-therapeutic study demonstrated safety and islet survival, but low packing densities to the point the diabetes product was sold to Neocrin Company. Scharp left the university and joined Neocrin in 1994 to develop this technology. But the Baxter macro-device became the early Neocrin focus due to its capacity to hold larger islet volumes than the hollow fibers. But, this Baxter device was returned to Baxter in 1996 after Neocrin was unable to confirm its utility in large animal studies. Neocrin turned its focus to the novel PEG CONFORMAL COATING technology developed by Jeff Hubbell and published excellent rodent results using encapsulated porcine islets. But attempts to translate these results in non-human primates stalled so that the company was closed in 1999. Scharp and Paul Latta were able to restart the company co-founding Novocell with a portion of the previous VC investors

providing the funding. This new start permitted the successful translation of the encapsulated conformal coatings from rodents to primates with the demonstration of a 20 month reversal of diabetes from the subcutaneous implants with only 30 days of low dose cyclosporine. These large animal results permitted the FDA approval of a corporate Phase I/II clinical trial for 12 patients with Type 1 Diabetes for Novocell. Yet, the company chose to close the trial and abandoned the encapsulation technology after the first two patient subcutaneous implants showed safety and many months of partial function but not curative responses. As the company changed its focus to developing human embryonic stem cells into insulin-producing cells, Scharp left Novocell. In 2006 he founded Prodo Laboratories to provide corporate and academic investigators human islets for research as well as develop a new class of islet specific tissue culture media and reagents. He also founded the Scharp-Lacy Research Institute that has an IIDP contract with the NIH and contracts with Prodo Labs to produce the islets for distribution to JDRF and NIH funded investigators. Prodo Labs funds its research and development by its revenues from these products without any outside funding. It was recently awarded a SBIR grant with a collaborator, Alex Gorkovenko, to develop a novel polymer that can be applied as a Minimum Volume Capsule for islets. These studies have returned Scharp to Islet Encapsulation development towards a clinical product that continues in Prodo Labs. In 2010, Scharp also founded Scharp Technologies producing adult human mesenchymal stem cell conditioned media as a primary component for skin care products through Stemage, owned by Edison Nation Medical.

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