CHAPTER 6

Pluripotent Stem Cell–Derived Islet Replacement Therapy for Diabetes

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THE UNMET MEDICAL NEED OF DIABETES

Diabetes is a worldwide epidemic, currently afflicting over 425 million people on the planet. The incidence of both type 1 diabetes (T1D) and type 2 diabetes (T2D) is increasing, and it is estimated that nearly 700 million people may have diabetes by the year 2045 (IDF website, 2017). In the United States alone, about 1.25 million people have T1D (ADA Website), and the healthcare economic burden of T1D amounts to approximately $14.5 billion in direct costs annually. Between T1D and T2D together, and including indirect costs, this figure may be as high as $1.3 trillion in the United States (Yang et al., 2013; Bommer et al., 2018). Extrapolating from the US statistics, it is likely that tens of millions of people are currently living with T1D worldwide. Collectively, these statistics portray a staggering healthcare crisis.

T1D is an autoimmune disease that occurs when a person's T lymphocytes mistakenly recognise the endogenous insulin-producing beta cells as foreign and destroy them. Without beta cells and insulin, extreme hyperglycaemia ensues, and prior to the advent of pharmaceutical insulin, discovered approximately 100 years ago, T1D was lethal within weeks to months of clinical symptom onset (Banting and Best, 1922). The introduction of insulin in the 20th century made living with T1D feasible, and since that time, numerous advances in insulin pharmacology, glucose monitoring, and insulin delivery, as well as other medications, have gradually improved the lives of patients suffering with this disease. However, there remain endless daily challenges of living with T1D including numerous finger sticks and the need to wear a continuous glucose monitor, estimation of the amount of insulin to take, dietary restrictions and self-administration of insulin (a potentially lethal drug), either by manual injection or a continuously worn pump. Furthermore, even with the best compliance on the part of type 1 patients, it is impossible to control blood glucose sufficiently to avoid the many secondary complications that arise. These long-term complications include cardiovascular and neurological problems, retinopathy, nephropathy and diabetic ulcers with the potential for limb amputations (Pickup and Williams, 2003). Moreover, because of the challenges of controlling

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blood glucose levels with pharmaceutical insulin, T1D patients are also always at risk of hypoglycaemia that can lead to coma or even death. Suffice to say that, given the magnitude of the problem, as well as the insufficient nature of the current standard of care, treatment of T1D remains a serious unmet medical need.

CELL THERAPY USING CADAVER-DERIVED ISLETS AND IMMUNE SUPPRESSION FOR TYPE 1 DIABETES: CLINICAL PROOF OF PRINCIPLE

A logical solution to this problem is to connect a continuous glucose monitor to the operation of an insulin pump via software. This concept, known as the closed-loop or artificial pancreas (AP), has received a great deal of attention, and tremendous progress has been made in recent years. Although clinical trials of various AP systems are underway, many challenges remain, and at least in their current manifestations, the AP systems are cumbersome and still require a great deal of user attention and sophistication (Bekiari et al., 2018).

What if, instead of using mechanical and electronic means to simulate the functions of the endocrine pancreas, one could harness the power of islet cells themselves? The beta cells and other cells of the pancreatic islets of Langerhans have evolved over millions of years to do the job of glycaemic regulation – monitoring glucose and secreting insulin and other hormones. If one could replace the cells that are destroyed or rendered dysfunctional by autoimmunity, with healthy new cells, and if one is able to protect the new cells from immune attack, a person with T1D might be functionally cured of the disease.

Indeed, there is a proof of principle that cell replacement therapy can be highly effective in the treatment of T1D. Islet transplant, originally pioneered in animal models and experimental clinical studies in the 1970s and 1980s (Nason et al., 1988), began to achieve notable clinical success in the 1990s (Scharp et al., 1990). In 2000, the development of steroid-free immunosuppression regimens, commonly referred to as the 'Edmonton Protocol' (Shapiro et al., 2000), greatly improved the clinical success rate, and islet transplant has evolved over the past decade to become a procedure that can render recipients insulin-independent, in some cases for many years posttransplant (Barton et al., 2012; CITR reference). For islet transplant, cadaver islets are harvested through a process of enzymatic digestion of donor pancreata (Linetsky et al., 1997) and administered to the patient via infusion into the hepatic portal vein. Islets lodge in the small capillaries of the liver, develop their own blood supply, and begin glycaemic regulation.

Although many patients have benefitted tremendously from islet transplant, two significant issues prevent its broad adoption as a standard procedure for T1D: (1) the supply of quality donor pancreata is extremely limited, and (2) patients receiving islet transplants need to take chronic immunosuppressive drugs to prevent immune destruction of the islets. Immunosuppressants have well-recognised risks and side effects, so the risk/benefit

ratio of the procedure and immune suppression regimen must be carefully evaluated. For some patients, the risks of chronic immunosuppression are outweighed by the benefit relative to their challenges associated with self-treatment for glycaemic control. In fact, for these patients the Centre for Islet Transplant Registry (CITR reference) suggests that the more limiting component preventing broad application of islet transplant is the scarcity of donor pancreata (OPTN, 2017). Clinical trials of cadaver islet transplants are advancing the approach towards approval by the FDA, and a Phase 3 trial recently concluded that 'islet transplant should be considered for patients with T1D and impaired awareness of hypoglycaemia in whom a stepped-care approach including current educational, pharmacological, and technological interventions, has failed to prevent life-threatening severe hypoglycaemic episodes' (Hering et al., 2016). This should facilitate reimbursement by health insurers, but until the supply issue is solved, it seems that islet transplant will remain a specialised procedure limited to only those patients with the most challenging glycaemic regulation.

DISCOVERIES ENABLING A RENEWABLE SOURCE OF ISLETS

Stem cells have two remarkable properties that make them intriguing players in the cell therapy picture. Stem cells (1) can proliferate, in some cases essentially indefinitely, while still maintaining their identity as stem cells and (2) can differentiate, given the proper cues, into other cell types that are within the given stem cell type's potential lineage domain. The *concept* of pluripotent stem cells, those cells that can give rise to all the different cell types of the body, is simply logical, and therefore as old as the study of developmental biology. Remarkably, in 1981, the first mammalian pluripotent stem cells, mouse embryonic stem cells (ESCs), were isolated from blastocysts and propagated in the laboratory (Evans and Kaufman, 1981; Martin, 1981). Over the following decades, mouse ESC proved to be extremely valuable in understanding mammalian developmental biology, including allowing for the first targeted gene knockout studies in mammals (reviewed in Brandon et al., 1995). With an understanding of the methods to isolate and propagate pluripotent stem cells also came the first real possibility that if cell differentiation pathways could be deciphered and recapitulated in the laboratory, then theoretically *specific cell types could be generated in virtually unlimited numbers* (due to the ability to limitlessly propagate the undifferentiated ESC prior to their directed differentiation to the desired cell type).

After much effort, almost two decades later, the first *human* pluripotent stem cells (human embryonic stem cells; hESCs) were derived and described (Thomson et al., 1998). In 1998, for the first time, the possibility of manufacturing unlimited supplies of specific human cell types, of all sorts, became a reality. Amongst the many theoretical therapeutic options to consider, one very obvious choice was to make replacement beta cells for the treatment of T1D. Several groups sought to pursue this goal, but arguably the

most uniquely focused and best-resourced effort in this regard was at a company named CyThera (later Novocell; now ViaCyte) in San Diego, California, USA. With the multi-million dollar budget that would be required to be successful, a team of approximately a dozen scientists collectively set to work on the task of figuring out the ideal 'recipe' for making pancreatic endocrine cells, with a focus on beta cells.

Using the developmental biology literature that described the gene markers and signalling pathways involved in the organogenesis of the pancreas, a theoretical framework for how to turn hESC into islet cells was mapped out. A process of trial and error began, testing various culture media and supplements, and specific protein as well as small molecule factors, with different concentrations and timings, iteratively moving the cells down the pathway towards the pancreas (Bastidas-Ponce et al., 2017). The process sought to replicate pancreatic organogenesis from the very earliest stages. The initial goal was just to drive the differentiation of pluripotent hESC to definitive endoderm lineage. Any cells that remained pluripotent, or instead became ectoderm or mesoderm, would only serve to create a mixed cell population, further complicating the process downstream and potentially adding a requirement for purification step(s). For the cell population to have a singular and clean identity, and be clinically useful, it was important to move down the differentiation tree (1) uniformly and (2) synchronously in a manner that was reproducible and also acceptable to regulatory authorities. These principles drove the studies, and the group made successive progress, first making high purity definitive endoderm (D'Amour et al., 2005) and later multiple intermediate endoderm cell types, eventually achieving high purity pancreatic endoderm (pancreatic progenitors and immature endocrine cells) (D'Amour et al., 2006).

While the insulin-producing cells generated using this initial protocol did not consistently respond to glucose in vitro, the team decided to test whether the hESC-derived pancreatic endoderm population might have sufficient potential and 'momentum' to become glucose-responsive insulin-producing tissue on implant. The pancreatic endoderm population contained pancreatic progenitor cells and polyhormonal endocrine cells, but many questions remained that could only be answered by studying the cells after implantation in an animal. Did the progenitors have the potential to further differentiate into endocrine pancreas including beta cells of the islets? Would the polyhormonal insulin-producing cells 'resolve' to make functional beta cells?

Work in the SCID-Bg immune knockout mouse model demonstrated that hESC-derived pancreatic endoderm produced by the San Diego team gave rise to islet tissue in vivo, including glucose-responsive insulin-secreting cells in sufficient numbers to be therapeutic (Kroon et al., 2008). Subsequent work determined that the mechanism by which pancreatic endoderm produced functional beta cell grafts was in vivo differentiation from pancreatic progenitors rather than 'resolution' of the polyhormonal phenotype (Kelly et al., 2011). Instead, the polyhormonal endocrine cells were found to become principally glucagon-expressing alpha cells (Kelly et al., 2011; Rezania et al., 2011), which are also considered a potential benefit for a therapeutic use.

Two other groups soon replicated the production of functional human islet grafts in mice and rats by implanting pluripotent stem cell–derived pancreatic progenitor populations (Rezania et al., 2012, 2013; Bruin et al., 2013, 2015), whereas numerous other groups produced similar populations in vitro but without robust in vivo analysis. Importantly, the presence of substantial numbers of PDX1 and NKX6.1 coexpressing cells appears to be a critical element for success (Rezania et al., 2013). Pancreatic progenitor–derived in vivo differentiated grafts contain the full complement of islet cells (Kroon et al., 2008; Rezania et al., 2012), display appropriate granule morphology by electron microscopy (Kroon et al., 2008; Rezania et al., 2012), are capable of responding to glucose within 5 min of injection (Schulz et al., 2012) and display a global mRNA expression and epigenetic profile nearly indistinguishable from primary adult human islets (Xie et al., 2013). Indeed, these cells have a similar immune profile and immunogenicity as beta cells in native islets (van der Torren et al., 2017). Moreover, the grafts contain all five endocrine islet cell types as assessed by immunohistochemistry, including appropriately functional alpha cells (Motte et al., 2014; Robert et al., 2018). Although the progenitor cells have the potential to become all of the three pancreatic lineages (duct, acinar and endocrine cells), empirically they show a clear propensity towards the endocrine lineage (Kroon et al., 2008).

Having determined the use of pancreatic progenitor cells had potential advantages over fully in vitro differentiated islets for cell therapy, and having developed a multistep manufacturing process, the ViaCyte team spent considerable effort over the next few years developing methods for scalable current good manufacturing practices (cGMP) manufacturing; standardised and custom quality control systems were established to comply with requirements for clinical application of the cells (Schulz et al., 2012; Schulz, 2015).

HOW ENCAPSULATION CAN CIRCUMVENT THE REQUIREMENT FOR IMMUNOSUPPRESSION

A specific hESC line was ethically derived under cGMP and is being used to manufacture pancreatic progenitors for the cell therapy product. These cells represent an allograft, and, as with donated organs, they need to be protected from alloimmunity. In addition, because the T1D recipients have autoimmunity to beta cells, the cells need to be protected against autoimmune destruction as well (van der Torren et al., 2017). Chronic immunosuppressive therapy is associated with elevated risk of infection, hypertension, chronic renal dysfunction and increased risk of specific malignancies, making its use undesirable in otherwise healthy T1D patients (Hering et al., 2016).

On implant, some cells adapt to their new environment and thrive, whereas others die, releasing their molecular remnants into the host. Host cells of the recipient, recognising the nonself molecules, mount an immune response to deal with the perceived

invaders. Although rejection may be initiated by the release of factors from the implanted cells, the process of allogeneic cell destruction requires direct cell–cell contact between host immune cells and the foreign cells (Wilcox et al., 2016). If host immune cells are physically prevented from making cell–cell contact, the allogeneic cells can survive. Accordingly, a variety of approaches have been developed in an attempt to isolate implanted cells from the host, while still allowing ingress of important nutrients to the implant from host vasculature, as well as egress of key factors out to host tissues.

Microencapsulation or Macroencapsulation?

In microencapsulation, typically, polymeric materials such as alginate, polycations and anions, agarose or polyethylene glycol (PEG) have been used to form coatings around individual cells or groups of cells, such as pancreatic islets, as an immune barrier (O'Sullivan et al., 2011). Microencapsulation approaches have been tested extensively, including a clinical trial of human cadaver islets for T1D, sponsored by Novocell, a predecessor company of ViaCyte (Scharp and Marchetti, 2014). For various reasons, some of which are described in Table 6.1 where the respective attributes of micro- and macroencapsulation are compared, it was decided that in the first test of pluripotent cell–derived pancreatic progenitors in human subjects with T1D, macroencapsulation would be utilised. The primary reasons included the ability to fully encapsulate with 100% integrity (therefore preventing immune destruction), better ability to vascularizse and removability.

Macroencapsulation began as a novel approach to protect allogeneic cells from host immune destruction pioneered by Baxter Healthcare, starting in the 1980s. After extensive preclinical investigation in various animal models, this effort was spun out as a company known as TheraCyte in 1999. Early investigations used a 'Boggs chamber', comprising circular pieces of semipermeable materials held together by a rigid ring. This format allowed investigators to readily test a variety of membrane materials, thicknesses and permeabilities for their effect on implanted cell survival and host tissue response. The result of this early research was the TheraCyte device, which comprises a cell-containing lumen surrounded by a cell-impermeable durable polytetrafluoroethylene (PTFE) membrane (Geller et al., 1997). By preventing cell–cell contact between host (immune and other) cells and the implanted cells within the device, the membrane ensures that allogeneic rejection of the implanted cells is prevented.

Baxter, and subsequently TheraCyte, along with other researchers, demonstrated protection of cells from allogeneic immune destruction in mice, rats and rhesus monkeys (Geller et al., 1997; Sweet et al., 2008; Tarantal et al., 2009) and later in sensitised hosts (Kumagai-Braesch et al., 2013). Importantly, clinical feasibility of macroencapsulation was demonstrated by the implantation of autologous and allogeneic primary parathyroid tissue, surviving as long as 12 months postimplant in human subjects (Tibell et al., 2001). In addition, the protection of allografts in a rodent model of diabetic autoimmunity was

Table 6.1 Comparison Between Common Encapsulation Technologies.

Microencapsulation	Macroencapsulation
Manufacturing	
Difficult to ensure 100% coating, which is required to block host immune cell contact	Device can be tested prior to introduction of cells to ensure integrity – prevent host cell ingress and implanted cell egress
Coating is formulated around cells/cell clusters as they are surrounded in droplets containing monomers, which are subsequently polymerised	Cells/cell clusters are typically introduced into the device lumen via a loading port, which is subsequently sealed
Design	
Smaller; coatings on micrometre scale	Larger, dimensions are several millimetres to centimetres per unit
Can 'tune' coating to effectively present a molecular sieve – potential to block diffusion of large molecules (e.g., proteins) – can provide xenoprotection	Protects against allogenic rejection Does not protect xenografts
The more obstructive this 'filter', the greater diffusion barrier to beneficial molecules such as oxygen, glucose and cell metabolites that can become toxic at high concentrations	Semipermeable, thin membrane allows diffusion of small and large molecules
Polymeric coating significantly increases volume of microencapsulated cell product and may limit implant site options	Device adds significant volume and footprint to implant Device geometry is constrained May limit implant site options
Clinical Implementation	
Long-term biostability of polymer coatings has not been established	Utilises biocompatible and biostable biomaterials with decades of human experience
Injectable coated cell suspensions are easy to introduce to certain anatomic locations	Introduced surgically; degree of invasiveness dependent on the implant location Device geometry must be compatible with anatomy of implant site
Difficult or impossible to ensure 100% removal of implanted cells that should be necessary for safety reasons	Supports surgical removal of therapeutic product, including implanted cells
Limited evidence of host vascularisation	Outer portions of devices can vascularise with networks of host vessels; promotes means of communication with systemic circulation

demonstrated (Lee et al., 2009). However, macroencapsulation devices of this design cannot support a xenogeneic cell source, as the xenorejection response is much stronger than allorejection, and implanted cells are believed to be suffocated by the fulminant inflammatory reaction (Brauker et al., 1996), a process that has been shown to require CD4+ T cells, but neither CD8+ T cells nor B cells (Loudovaris et al., 1996). For this reason, preclinical studies using human cells have to be performed in immunocompromised animals.

Based in part on these preclinical and clinical data, ViaCyte elected to switch from the PEG microencapsulation approach developed by Novocell to macroencapsulation. Questions around the potential safety risk of introducing a pluripotent stem cell–derived cell population into patients and the need to protect these allogeneic cells from immune rejection without adding immunosuppressive therapy to patients helped drive this decision. This strategic change was implemented to be compatible with the allogeneic pancreatic progenitor cells source-derived from pluripotent stem cells. This novel cell-device combination therapy requires not only engraftment and implanted cell survival but the cells also need to differentiate along target pathways and mature into functional pancreatic endocrine islet cells.

Preclinical studies of unencapsulated pancreatic progenitor cell implants demonstrated that significant number of cells matured into the target islet cell populations including alpha, beta, delta, epsilon and gamma cells which produced glucagon, insulin, somatostatin, ghrelin and pancreatic polypeptide, respectively (Kroon et al., 2008; Kelly et al., 2011). Some had hypothesised that macroencapsulation of pancreatic progenitor cells could introduce issues including poor engraftment, limited vascularisation and oxygenation leading to poor cell survival and function, off-target differentiation in vivo, an unacceptable lag between rise of blood glucose and insulin release resulting in 'insulin stacking' and hypoglycaemia and ultimately insufficient production of insulin. However, a series of studies in immunodeficient rodents demonstrated that macroencapsulation in either TheraCyte's or ViaCyte's prototype devices revealed clear safety and functional benefits. A surprising finding was that macroencapsulated implants achieved functional levels more rapidly after implant than unencapsulated grafts, although the underlying mechanism is not well understood (ViaCyte unpublished data). However, maximal function of the encapsulated implants is capped compared with unencapsulated, unconstrained grafts, likely due to physical constraints of the device lumen which limit cell proliferation. Another observed benefit is constrained growth of nontarget cell types, such as ducts, that may form in the maturing pancreatic progenitor tissue. Duct cells are not associated with any known safety issue directly but theoretically could cause graft enlargement while delivering no benefit (Kroon et al., 2008).

Collectively these observations drove a process of device optimisation that (1) involved selecting material configurations that maximised host tissue healing and vascularisation, (2) accommodated in vitro formulation of the combination product and (3) balanced the mechanical forces exerted by the implanted cell population with the device stiffness and host interstitial tissue pressures. For the latter, a particular

Figure 6.1 EN20 (20-μL nominal lumen volume) Encaptra device used in preclinical rodent studies and the human sentinel units (top). Larger EN250 (250-μL nominal volume) dose-finding device (bottom). The Encaptra device is made of medical grade plastics including an expanded polytetrafluoroethylene cell-impermeable membrane. The EN20 is approximately 11 mm × 21 mm, whereas the EN250 is approximately 30 mm × 90 mm; both are less than 1 mm thick. *(From ViaCyte.)*

force balance was established that leads to a consistent and predictable implanted cell lumen thickness, controlling total cell mass and resulting in a long-term steady state condition for a mature implant. Under normal conditions, this steady state can be monitored via ultrasound imaging to ensure that unexpected cell expansion is not occurring, thus serving as an inexpensive and noninvasive clinical diagnostic tool. It is likely that different implanted cell populations may exert different forces on a macroencapsulation device, and that the device design may need to be adjusted depending on the cell type being delivered. This development work ultimately resulted in the Encaptra cell delivery system family of macroencapsulation devices, two of which are shown in Fig. 6.1.

A study directly comparing engraftment and maturation of micro- and macroencapsulated pancreatic progenitor cells showed that microencapsulation does not result in significant human insulin production, whereas macroencapsulated pancreatic progenitors out-performed unencapsulated grafts (Motte et al., 2014). Studies with other sources of human pluripotent cell–derived pancreatic progenitors, implanted in TheraCyte devices, have also demonstrated significant insulin production (Rezania et al., 2013; Bruin et al., 2013, 2015).

Limitations of Encapsulation

In pancreatic development, immature progenitor cells that will ultimately become islets secrete growth and angiogenic factors that cause blood vessels to infiltrate and become integral to their maturing structures. The result is highly vascularised islets wherein each

alpha, beta or delta cell is typically within 2–3 cell diameters of a blood vessel (Cleaver and Dor, 2012). The close proximity of capillaries with beta cells is important to ensure that the cells rapidly sense blood glucose levels and have enough oxygen available to release the required amount of insulin, and that released insulin can be distributed rapidly to the rest of the body.

By its very nature, encapsulation puts a diffusion barrier between the implanted cells and host vasculature. The rate of diffusion over such a barrier is primarily dependent on the permeability (or porosity) of the material, size of the diffusing molecule and distance (thickness).

Beta cells require high concentrations of oxygen to produce insulin as reflected by the abundant vascularisation of the islets of Langerhans. To maintain viable functioning beta cells inside a device, the diffusion distance from host blood vessels needs to be minimised, while the permeability of the encapsulation material is maximised. If large quantities of oxygen cannot diffuse across an encapsulation barrier, the beta cells will be unable to manufacture insulin in adequate quantities to control blood glucose. At low oxygen levels, beta cell death will occur (Emamaullee et al., 2006).

Thus, the thickness of the encapsulation barrier becomes important, along with the geometry of the cell and device design, to minimise the distance between host blood vessels and the implanted cells. For microencapsulation technologies, the coating process must be highly controlled to limit the coating thickness, which can exceed 100 μm, presenting a significant barrier for diffusion of oxygen, glucose and insulin. This consideration must be balanced against the requirement to have a sufficient amount of encapsulation material to achieve a 100% conformal coating. In the absence of a complete conformal coating, host immune cells can come into direct communication with implanted cells, resulting in their destruction via innate and/or adaptive mechanisms.

Macroencapsulation devices face a similar diffusion challenge. Computer simulations of beta cell oxygen demand required just for cell survival relative to capillary supply (Buchwald, 2011) predict that oxygen diffusion within an islet cell mass is limited to approximately 100 μm from the capillaries. Significantly higher oxygen concentration is required to support effective insulin production by beta cells, which therefore must be located even closer to the oxygen source. These predictions are supported by preclinical observations of explanted VC-01 grafts – cells within the device together called the VC-01 combination product candidate – wherein the implanted cell mass, on maturation, typically reaches approximately 200 μm in thickness. This constraint limits geometric options of macroencapsulation devices, with a flat sheet configuration being the most straightforward to implement. Based on this consideration, Weir (2013) believes that providing sufficient beta cell mass to achieve insulin independence may require devices with a surface area on the order of 400 cm^2, which if correct would presumably require some design reconfiguration to be most practical.

Other limitations of encapsulation need to be considered when designing a system for a given cell application (Vaithilingam et al., 2017). If a xenogeneic cell source is being considered, then it may be necessary to block the diffusion of certain molecules, typically proteins, from escaping the implanted cell mass and entering host tissues to avoid initiating a violent rejection process. Through choice of the microencapsulation polymer mix and optimising polymer concentration, the coating can effectively be designed as a molecular sieve to prevent the diffusion of large molecules such as these xenogeneic proteins. However, by interfering with the diffusion of large molecules, there is an unavoidable impact on movement of small molecules as well. Furthermore, limiting oxygen diffusion of glucose and other nutrients can have a significant impact on health and function of an implanted cell population (Geller et al., 1997). Properly designed microencapsulation may be capable of xenoprotecting implanted cells while maintaining a sufficient nutrient environment to support viability and function.

In general, the membranes implemented in macroencapsulation devices are more porous, and therefore less selective or tunable, compared with microencapsulation polymer configurations. This limitation allows free diffusion of even large molecules, including xenogeneic proteins that may elicit a strong host immune response. Preclinical studies of immunoisolating cell chambers, TheraCyte and Encaptra devices containing xenograft tissue have demonstrated that a fulminant inflammatory response occurs rapidly after implantation (Brauker et al., 1996). Implanted cells die within a matter of a few days or weeks, too soon to be a result of a coordinated adaptive immune rejection response, but more likely because the graft is starved of nutrients. Although some macroencapsulation devices have the demonstrated ability to protect against allogeneic destruction, they typically are not suitable for xenograft applications.

FOREIGN BODY REACTION AND VASCULARISATION

Any material introduced into the body will be met by an array of responses that have evolved to protect an individual from foreign bodies. The nature and severity of the foreign body reaction can range from an acute, minor local irritation to severe, chronic inflammation that prevents healing, and which can be exacerbated by release of cytotoxic factors or other irritants. Indwelling biomaterials may also serve as a colonisation site for microbes, providing a local environment that interferes with the effectiveness of antibiotics.

Normally a host isolates foreign materials by forming a foreign body capsule (FBC) around the material. Initially, surrounding tissues become acutely inflamed because of the release of factors on injury. Inflammatory molecules trigger recruitment and activation of macrophages and immune cells, followed by early angiogenesis to feed these cells as they gather to fight the material or insult. Host cells also secrete proteins to create an extracellular matrix that begins to form a fibrotic capsule over time. Eventually, further

fibrosis and shrinkage of the resulting capsule into scar tissue is accompanied by a reduction of vascularity (Geller et al., 1997).

Preclinical studies of macroencapsulated human pancreatic progenitor cells implanted in immunocompromised rodents have demonstrated that the foreign body reaction differs significantly from that observed with medical devices comprising nonbioactive materials. Some factors that may impact local tissue inflammatory reactions perioperatively include the following:

- surgical trauma and related healing – damage to connective tissues, destruction of host cells and release of their contents
- biomaterials in device – related to material and its structure or configuration. Initially, the inflammatory response may be significant, but with nonbioactive, biocompatible and biostable materials, this typically subsides over time, resolving into a low level of chronic inflammation
- release of factors from normal metabolism of implanted cells – some will not be recognised as foreign and will not elicit a significant response; others may be recognised as nonself and activate an allogeneic immune response. The level of response is likely affected by the magnitude of nonself molecules released into surrounding host tissues
- diffusion of antigenic and damage-associated molecules into host tissues as a result of cell death due to implant-related acute stress, apoptotic processes or natural cell death and turnover

While the local foreign body response to a nonbioactive medical implant typically resolves to a fibrotic capsule over time, a combination product containing living cells appears to result in a different type of capsule. ViaCyte's PEC-01 cells, much like the pancreatic progenitor cells in a developing embryo, make factors that can facilitate host angiogenesis. In preclinical rodent studies, the capsule becomes highly vascularised and integrated with the outer portions of the macroencapsulation device (Fig. 6.2) (ViaCyte unpublished data).

Host vessels appear to grow towards cell-containing areas of the device's cell lumen. After 3–4 months, a dense plexus of capillaries covers the surface of the immunoprotective membrane, overlying implanted cells that have differentiated into mature islet cells types. The implanted progenitor cell population coalesces and proliferates to form a large pancake islet-like structure within the device lumen (Fig. 6.3). Longer term studies, taken out to the life span of the immunocompromised mice, have demonstrated that the capsule remains vascularised, and grafts remain functional, to beyond 12 months after implantation (ViaCyte unpublished data).

PRODUCT MANUFACTURING

When designing a product for humans, many requirements impact the design of the macroencapsulation device, cell processes and the configuration of the resulting combination product and its packaging. With patient safety as the highest priority, the implanted

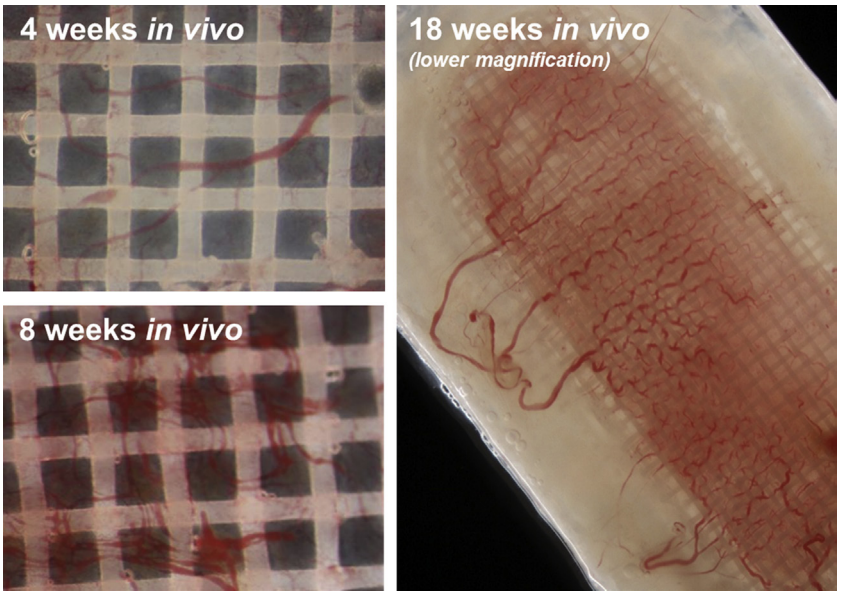

Figure 6.2 Host vascularisation of Encaptra devices containing pancreatic progenitor cells in SCID-Bg mice. After 8 weeks, vessels are intercalated amongst the outer mesh layers of the device (bottom left). After 18 weeks, a dense network of small blood vessels covers the device membrane in areas where implanted cells are present; these are fed and drained by a small number of larger vessels (right). *(From ViaCyte.)*

cell product must first be prepared under aseptic conditions with cGMP controls in place. Cell expansion, controlled differentiation and harvest are performed in aseptic cleanroom environments prior to cryopreservation of the drug substance (DS). Once frozen, safety and quality testing can be performed prior to release of DS for further processing into the final combination product (drug product; DP).

Separately, macroencapsulation devices are manufactured in a cleanroom environment under medical device cGMP controls utilising biocompatible, biostable, polymeric biomaterials and custom equipment. Following quality control testing, devices are packaged and terminally sterilised.

For ViaCyte's combination cell-device product candidate, formulation of the DP consists of several stages, beginning with thaw of DS (pancreatic endoderm cells) under tightly controlled aseptic conditions. To maximise the number of healthy cells delivered to the patient, DS is cultured for several days to reinvigorate those that have survived the cryopreservation process and eliminate those that have not. Cultured DS cells are harvested and aliquoted into doses. Formulation of DP includes aseptic loading of DS into encapsulation devices utilising in-process packaging that is designed to prevent contamination and facilitate cell delivery into device lumens. Device ports are sealed, and the cell-filled devices are placed into primary sterile packaging with cell storage medium.

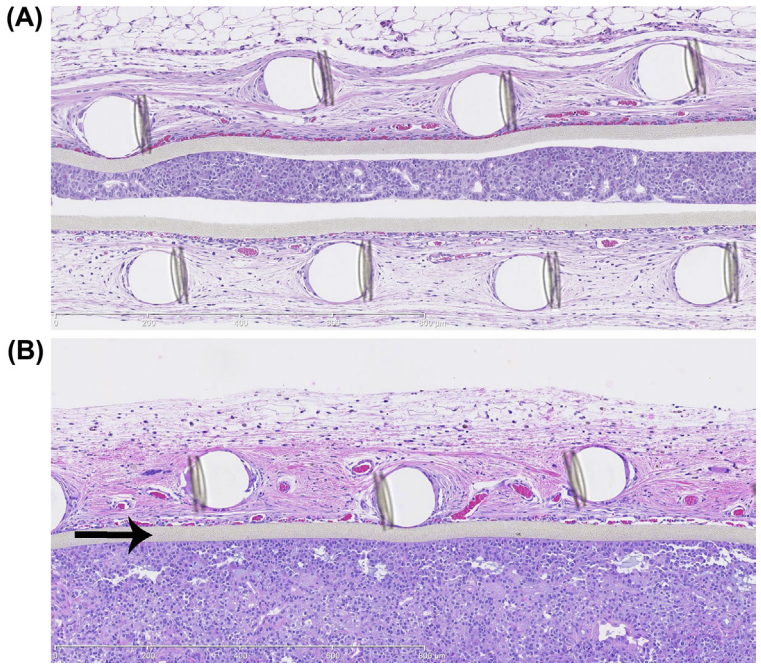

Figure 6.3 (A) Haematoxylin and eosin stain of Encaptra-encapsulated pancreatic progenitor graft after 4 weeks in an SCID-Bg mouse. Even at this early time point, significant host vasculature is seen adjacent to the semipermeable device membrane. Gaps between implanted cells and the membrane are histological artifact, and the circles represent cross sections of the structural mesh of the device. (B) At 24 weeks, cells have matured and coalesced into a pancake-like cell mass of mature islet cell types (below the semipermeable membrane identified with *arrow*). The host surface of the membrane is covered with blood vessels which extend through the foreign body capsule. *(From ViaCyte.)*

While a combination product may be considered a biologic by regulators, the physicians utilising the product are more likely to see it as a physical device that needs to be handled carefully, but is otherwise an implantable medical device. Operating room procedures and staff have established desired and mandatory requirements for implantable medical devices that need to be considered when designing the macroencapsulation device and its associated packaging, labelling and accessory tools. The product must be designed from the user's perspective, taking predicate products and procedures into account where possible and leveraging available surgical technologies and instruments. As an example, it may be desirable to rinse the product to dilute excipients prior to implantation. Procedures utilised in cardiac surgery to serially rinse bioprosthetic heart valves, intended to dilute cytotoxic preservatives, can be implemented. Due to the high cost of operating room time and to minimise the risk of contaminating product, it is important to streamline product preparation and implantation procedures.

Many cell therapies face a challenge in identifying the best anatomical site for administration and method of delivery, and indeed for some only a specific location may be efficacious. In the case of T1D, patients inject pharmaceutical insulin subcutaneously and are able to successfully manage their blood glucose. This observation demonstrates an important proof of concept that for an insulin-delivering cell therapy, an ectopic site such as the subcutaneous space, omentum (via minimally invasive surgery) or other less invasive locations compared to the pancreas should be effective as long as the product is sufficiently integrated with the patient's vascular system, and other requirements are met (Giraldo et al., 2010). Indeed, subcutaneous implants of macroencapsulated pancreatic progenitor cells in mice have shown good cell survival and successful engraftment. Ultimately, these grafts have been able to control blood glucose to human set points for up to a year, the life span of these animals. Alternate sites exist, but as they become increasingly invasive they acquire elevated risk to patients for the surgical implantation and explantation procedures, especially if the product has become highly integrated with the patient's tissues.

PRECLINICAL STUDIES AND REGULATORY SUBMISSION

Regulatory agencies typically classify medical products into one of the three categories: drug, biologic or medical device. An increasing number of medical products incorporate two or more of these elements. These combination products are regulated based on their primary mode of action (PMOA). In the case of a cell therapy–based combination product to treat insulin-dependent diabetes, the implanted cells (biologic) produce insulin (drug) and other molecules in response to biological cues from the host and are contained with a macroencapsulation device (medical device). Because the PMOA is the activity of the cells, this product is treated as a biologic, but all relevant medical device regulations must also be satisfied. Implementing appropriate quality systems in compliance with cGMP for combination products is challenging, sometimes without clear guidance available from regulators as to how the varied regulatory requirements should be implemented and often involves development of custom assays and quality systems. It is the responsibility of each company or product development group to decide how to approach regulatory compliance.

With the product concept settled following years of research studies, ViaCyte worked with the FDA through Pre-pre-IND (2009) and Pre-IND (2012) meetings to establish a package of preclinical studies that would provide sufficient data to reasonably assure safety and efficacy in animals prior to human testing. Although the field is nascent, a fairly consistent set of good laboratory practice (GLP) studies is emerging for pluripotent cell–derived products (Nasis et al., 2013). ViaCyte performed ISO 10993 testing for the Encaptra macroencapsulation device and submitted a Device Master File (MAF) in July 2014. The investigational new drug application (IND) was submitted separately,

referencing the MAF, and described the now standardly manufactured cGMP pancreatic progenitor cell population, known as PEC-01 cells and their combination with the Encaptra device – cells within the device together called the VC-01 combination product candidate.

FIRST CLINICAL STEP – THE STEP ONE TRIAL

The Phase 1/2 clinical trial, known as the STEP ONE trial (Safety, Tolerability and Efficacy of VC-01 Combination Product in Type One Diabetes) was launched in September 2014, and the first subject was implanted with the VC-01 product candidate in October 2014. The STEP ONE trial is an open-label dose-escalation trial with two planned cohorts of patients. The first cohort is designed to evaluate safety, and patients in this cohort receive what is calculated to be a subtherapeutic dose of cells. Patient accrual in this trial is intentionally gradual, so that each subject can be evaluated for several weeks before the next one is implanted. In this way, the team is also able to implement improvements along the way, as new information is continually emerging regarding this type of product and approach that have never been tested before. The trial includes both 'dose-ranging' VC-01-250 units, which could potentially deliver therapeutic amounts of cells, and 'sentinel' VC-01-20 units, equivalent to those used in rodents. The sentinel units are explanted periodically to allow histological assessment of product engraftment, including determination of cell survival within Encaptra, phenotype of surviving cells (e.g., pancreatic progenitors becoming endocrine pancreas), any formation of a capsule around the product, the potential for presence of immune cells on or around the product and vascularisation of the product by the patient. Patients in the first cohort are receiving up to two VC-01-250 units and between four and six VC-01-20 sentinel units. Once safety is established, the perioperative procedures have been optimised for maximum engraftment, and the data are reviewed by an independent Data Safety Monitoring Board, then enrolment in a second cohort may begin. The current plan is for patients in the second cohort to each receive six VC-01-250 units and up to two VC-01-20 sentinel units. The trial is expected to enrol a total of approximately 50 patients, and implants are expected to remain in the patients for up to 2 years. The first data from the STEP ONE trial were presented at the American Diabetes Association 78th Scientific Sessions in 2018. In summary, these data showed that the VC-01 product candidate has been safe and well tolerated, the Encaptra device appears to be immune protective as designed, and PEC-01 differentiation into β-cells and long-term cell survival has been observed up to 2 years when VC-01 units are well vascularised. However, vascularisation and engraftment were inconsistent and not sufficiently robust due to the foreign body response, and therefore modifications are being made to the Encaptra device before additional subjects are implanted (Henry et al., 2018).

WHAT ABOUT USING INDUCED PLURIPOTENT STEM CELLS AS SOURCE MATERIAL FOR CELL THERAPY?

In 2006, the novel concept of generating induced pluripotent stem cells from adult (nonstem) cells was first described (Takahashi, Yamanaka, 2006). This opens up for the field of cell therapy the possibility of generating unlimited pluripotent cells, and therefore theoretically substantial amounts of any tissue, from anybody. The issues of alloimmunity and allorejection of implanted therapeutic tissues would likely be circumvented if the induced pluripotent stem cells (iPSC) were generated from an autologous source. However, for a treatment of T1D, it is expected that autoimmunity would still represent a substantial issue.

Additionally, to the extent that some may have concerns around the use of human embryos (albeit preimplantation blastocysts ethically derived and otherwise destined for destruction or cryopreservation) as the source material for hESC, iPSC avoid this issue by typically using a small biopsy or blood draw as source material for their derivation.

For these various reasons, several groups have endeavoured to generate pancreatic progenitor cells and endocrine pancreas from iPSC (Rezania et al., 2014; Pagliuca et al., 2014), including ViaCyte scientists in collaboration with the Nestlé Institute of Health Sciences, SA, Lausanne, Switzerland (Haller et al., 2018). While modest adjustments were made to the differentiation protocol to accommodate this different starting material, pancreatic progenitor populations generated from iPSC appear and behave very similarly to those populations derived from hESC.

While there are theoretical reasons that iPSC could be a preferred pluripotent starting material for cell therapies, several practical and empirical observations suggest that caution should be taken with this approach. From a manufacturing perspective, if one wanted to take advantage of the ability to avoid immune suppression by using autologous cells, at least one novel cell line would need to be derived, expanded, banked and fully characterised for every patient to be treated. This labour requires a substantial amount of time and is far more cumbersome than using a single hESC line to treat essentially every person with any given disease. There have been suggestions that a new iPSC line would not necessarily need to be derived for every single recipient, but instead a library of iPSC lines, covering the vast majority of human leukocyte antigen (HLA) type combinations, could be created to match the vast majority of patients (Wilmut et al., 2015). However, it is important to note that achieving an HLA match does not obviate the need for immunosuppression because several other major and minor histocompatibility loci are not matched and are sufficient to generate immune recognition of the allogeneic cells (Sordi et al., 2017). It is also possible that cells derived from autologous iPSC may display differential immunogenicity depending on the cell type in question (Zhao et al., 2015).

Recent research has indicated that despite their similar behaviour and appearance, iPSC are not biologically the same as hESC. iPSC can have genomic issues not seen in

hESC, as they retain some of the history of the adult cell source from which they are derived, be they somatic mutations or epigenetic imprints acquired on developmental differentiation (Peterson and Loring, 2014). iPSC do not have the benefit of the natural 'resetting and refreshing' of the genome and related subcellular components that hESC bring from their recent passage through the zygote stage of the life cycle.

Nevertheless, enthusiasm for iPSC-derived products is high, and in 2014, the first iPSC-derived cells, retinal pigmented epithelial cells, were administered to a human subject, a patient with macular degeneration (Cyranoski, 2014). However, in 2015, this clinical trial of iPSC-derived cells was halted as a concerning genetic mutation was identified in an iPSC line created from the second subject's cells. This mutation could have been preexisting in the tissue used to derive the iPSC, or alternatively introduced into the cells during the derivation or propagation process, with potentially greater implications (Andy Coghlan, 2015; Mandai et al., 2017). In any case, these observations beg the question of what is the appropriate level of required characterisation of iPSC, and what data on specific iPSC lines need to be generated before one fully understands the variability and risks therein.

CONCEPTUAL ADVANTAGES OF USING PANCREATIC PROGENITOR CELLS AS COMPARED WITH ADULT ISLETS OR PURER POPULATIONS OF BETA CELLS

While the original notion in the field was obvious and simple – beta cells are lost in T1D, so the replacement cells should be beta cells – as described above, the ViaCyte team found that pancreatic progenitor cells worked well in preclinical studies and decided to proceed with that population for product development and the first clinical trial. In parallel with the VC-01 product development, several groups, including scientists at ViaCyte, continued to work towards a protocol to differentiate pluripotent cells into beta cells that are glucose responsive in vitro. In retrospect, we now know that this major accomplishment took many years beyond generation of the first in vivo–responsive cells, yet eventually multiple groups including ViaCyte described methods for manufacturing more mature beta-like cells in vitro (Rezania et al., 2014; Pagliuca et al., 2014; Agulnick et al., 2015; Russ et al., 2015).

In spite of this, for several reasons we believe that a pancreatic progenitor population remains a preferable option for a cell therapy product (Schulz, 2015; Table 6.1). These considerations include the following:

- Fully mature beta cells require an established oxygen supply. PEC-01 cells, being less mature, are expected to better tolerate a low oxygen environment and thus may have a greater likelihood of surviving in the initial oxygen-deprived environment encountered on implant. This hypothesis is supported by studies showing that engraftment of mature islets in macroencapsulation devices requires preimplantation and 'prevascularisation' (Sörenby et al., 2008), whereas macroencapsulated pancreatic progenitors do not require prevascularization (Rezania et al., 2013, ViaCyte, unpublished observations)

- Multiple endocrine hormones can provide benefit in T1D. In the animal models (results in human subjects have not yet been described), pancreatic progenitor cells generate all of the different islet cell types (alpha, beta, delta, epsilon and gamma) (Kroon et al., 2008; Rezania et al., 2013). While the absence of insulin is most obvious, beta cells are not the only dysfunctional islet cells in T1D (Gerich et al., 1973; Bonnet-Serrano et al., 2018). By providing the full complement of islet cell types, the product candidate has the potential to deliver the other pancreatic hormones including amylin, glucagon, somatostatin, ghrelin and pancreatic polypeptide, which work in concert to control blood glucose levels (Pickup and Williams, 2003). Augmenting or replacing the functions of these other cell types may provide additional benefit beyond replacing beta cells alone; most notably, delivering glucagon-secreting alpha cells may provide important protection against dangerous hypoglycaemic episodes (Yang et al., 2013).
- Beta cells may function better in their full islet 'community'. Including all of the different islet cell types may allow them to exist in a more natural physiologic and anatomical environment than beta cells alone could. The islet cells likely communicate via paracrine signals as well as direct cell–cell interactions including gap junctions (Meda, 2013). It is not known whether, and is even unlikely that, beta cells alone would be capable of providing the exquisite degree of glycaemic control that normal healthy islets provide.

At a practical level, several of these remain conceptual advantages over a hypothetical pure mature beta cell population generated in vitro, which has yet to actually be generated. That is, the 'more mature' cell populations recently described by various groups actually include all of the endocrine pancreatic cell types, although they do show skewing of their ratios towards beta cells (Rezania et al., 2014; Pagliuca et al., 2014; Agulnick et al., 2015).

It also has been suggested that by implanting cells that are closer to their terminally differentiated beta cell phenotype, the latency to graft function will be shorter. However, multiple factors contribute to the latency. These include (1) how close the cells are to their mature endocrine phenotype when implanted, (2) the dose of cells (fewer cells can expand and reach the levels of a larger dose; it just takes more time (ViaCyte unpublished observations), (3) the time and amount of graft vascularisation and (4) presumably the transplant site, which can affect degree of engraftment. At least in terms of what has been reported, the more mature populations do not show significant biologic function immediately on implant (summarised in (Schulz, 2015; Table 6.1)), and indeed the original report of pancreatic progenitor cells demonstrated robust glucose-stimulated human C-peptide in mouse serum within just 28 days of implant (Kroon et al., 2008). Even in the context of human cadaver islet cell transplant, there is some delay between implantation and full function – again likely due to the time it takes for vasculature to develop.

Lastly, for patients who have been living with T1D for several years, even decades, one might anticipate that waiting a few weeks or months for the implanted cells to take over their glycaemic control would be negligible in the larger scheme.

Instead, other considerations, including pharmacoeconomics, contribute to the decision-making more so than postimplant latency to function. These include the time and complexity of the manufacturing process. With more time and more process steps, the manufacturing will cost more and have more opportunities for process errors and batch failure.

OTHER APPROACHES TO ADDRESSING THE PATIENT'S IMMUNE SYSTEM

Once the VC-01 STEP ONE clinical trial was underway, a second version of the product, known as the VC-02 product candidate, was also prepared for clinical testing. The VC-02 product candidate delivers the same PEC-01 cells as VC-01, but in a device that has been engineered to allow direct vascularisation of the implanted cells. In this way, the foreign body reaction is circumvented, and a robust graft can develop. However, as with cadaver islet transplants, because of the direct cell–cell contact between the patient's immune system and the allogeneic PEC-01 cells, continuous pharmacological immunosuppression is required. Because pharmacological immunosuppression has associated risks and side effects, the VC-02 product candidate is being explored in T1D patients who are in more dire circumstances with their disease: those with hypoglycaemia unawareness, recurrent severe hypoglycaemic episodes and extreme glycaemic lability. The IND and clinical trial application (CTA) for VC-02 clinical testing were allowed in the United States and Canada, respectively, and the first patients were implanted in mid-2017. While these high-risk T1D patients are a more limited population for treatment than VC-01 is addressing, developing the VC-02 product candidate represents a strategy to expeditiously get the PEC-01 cell replacement therapy to those patients that have the greatest clinical need. Should the initial stages of the trial yield positive safety and efficacy data, the VC-02 product candidate might also be tested in diabetic patients who have received a kidney transplant for example. In this case, the patients are already on chronic immunosuppression to protect their transplanted organ, so presumably the addition of an efficacious cell therapy without additional immunosuppressive drugs required will represent only a clear upside to these patients.

Allogenic cell replacement of islet tissue, and of endocrine tissues in general, is rather uniquely compatible with the approach of encapsulation because the endocrine gland can be delivered into ectopic anatomical locations and need not integrate into an existing organ architecture. However, many other diseases may be effectively treated with allogenic cell replacement but are not compatible with encapsulation strategies (e.g., cardiac, neural, ophthalmic, etc.). As a result, we expect that the development of improved immunosuppressive and modulatory drugs, adjunctive cellular therapies (e.g., regulatory T cells) and genetic engineering approaches will remain an intense area of investigation with the ultimate goal of achieving immunological tolerance to stem cell–derived grafts (Talib et al., 2015). As these fields progress, we expect that an effective way of establishing

tolerance to an islet replacement graft will be achieved and that it can overcome certain constraints of encapsulation, principally related to a lack of direct vascularisation by the host and the potential for negative impacts on graft function and longevity in part associated with reduced oxygen delivery.

In the nearer term, the evaluation of lower doses and different combinations of approved immunosuppression drugs may permit the establishment of operational tolerance to islet replacement grafts, a situation that is not uncommon for liver transplantation on immunosuppression withdrawal (Liu et al., 2013). Indeed, a negative impact of calcineurin inhibitors on the relative balance of effector versus regulatory T cells has been described (Akimova et al., 2012). Calcineurin inhibitor–free regimens, including the use of new biological agents to effect costimulatory blockade, represent avenues of interest for clinical study with stem cell–derived islet replacement.

In addition, new genome editing tools (e.g., zinc-finger nucleases, TALEN, CRISPR/Cas) have greatly improved the efficiency and expanded the cell types in which the human genome can be precisely engineered (Vasileva et al., 2015). These advances make genetic engineering strategies to immune evasion and immune protection feasible. Indeed, several reports of the genetic engineering of HLA expression or transgene-based expression of costimulatory pathway genes in pluripotent stem cells (Riolobos et al., 2013; Rong et al., 2014) and primary cell types (Zaldumbide et al., 2013; Li et al., 2014) have already been published.

Most of these strategies have leveraged the growing understanding of how cancer and virally infected cells (autologous cells) evade immune recognition and destruction. It will be interesting to see whether these approaches will work sufficiently to protect specific cell types present in a therapeutic allograft. Moreover, it is important to consider that implanting a stem cell–derived population that has permanent immune tolerance or immune-evasion properties will increase the need for an in-depth preclinical assessment of tumorigenicity and safety assurance.

PERSPECTIVES

A tremendous amount of research and development has led up to clinical testing of pluripotent stem cell–derived human islet tissue. Several possible cell types might also be investigated, but allogeneic human pancreatic progenitors appear to have certain advantages relative to others (Hayek and King, 2016). Several administration strategies are also being investigated presently (Vaithilingam et al., 2017). These include the deployment and immune protection via macroencapsulation with an immunoisolating cell–impermeable membrane, pharmacological suppression of immunity and genetic engineering of the source cells to render them immune evasive. Likely, these will find their respective places in the market and biomedical history, as the clinical research unfolds and commercialisation is realised.

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