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# Cell Replacement Therapy: The Rationale for Encapsulated Porcine Islet Transplantation

Stephen J. M. Skinner, Paul L. J. Tan, Olga Garkavenko,  
Marija Muzina, Livia Escobar and Robert B. Elliott  
*Living Cell Technologies (NZ) Ltd*  
*New Zealand*

## 1. Introduction

Among the problems posed by chronic diseases today, one of the most daunting is that posed by diabetes mellitus, which is a very significant public health problem resulting in substantial morbidity and mortality (American Diabetes Association, 2011). With the increasing life-expectancy of the world's population, increasing exposure to environmental trigger factors, the rising incidence of obesity, and lifestyle changes such as unhealthy diets and decreased physical activity, the prevalence of diabetes has risen dramatically over recent years and is now reaching epidemic proportions globally. This rapid increase is a significant cause for concern, with an additional 7 million diagnosed each year (International Diabetes Federation, 2011). Diabetes is now the fourth or fifth leading cause of death in most developed countries (International Diabetes Federation 2000). Globally, it is estimated that more than 200 million adults now have diabetes and this number is expected to increase alarmingly in the coming decades. By the year 2025, it is estimated that almost 333 million people will have the disease (International Diabetes Federation 2006).

Type 1 (insulin-dependent) diabetes accounts for 5% to 10% of all diagnosed cases. In 2003, approximately 4.9 million people (0.09% of the world's population) were estimated to have type 1 disease, with Europe having the highest number of sufferers (1.27 million) followed by North America (1.04 million) and Southeast Asia (0.91 million). The highest prevalence of type 1 diabetes was in North America (0.25%) followed by Europe (0.19%) (International Diabetes Federation 2006). In 2002, there were an estimated 0.9 to 1.2 million people with type 1 diabetes in the USA (American Diabetes Association 2006). The incidence in recent years may have accelerated alarmingly as shown in a recent study from Finland where the rate increased from 31.4 per 100,000 per year in 1980 to 64.2 per 100,000 per year in 2005 (Harjutsalo et al, 2008).

In New Zealand, the incidence of type 1 diabetes has doubled in the last 15 years, reflecting international trends. In 2003, the estimated prevalence of type 1 disease among the population aged <25 years was 0.18%, with the total number of sufferers in this age range numbering 2540. The majority, 85% (2158 people), were of European descent, while 9% were Maori, 2.9% were Pacific peoples, and 3.0% were Asian (Wu et al. 2005).

Although the life-expectancy of patients with type 1 (insulin-dependent) diabetes mellitus has vastly improved since the introduction of insulin, the ability of insulin injections to

reliably prevent wide fluctuations in blood glucose levels is often inadequate and many patients develop complications of the disease. These complications cause considerable disability and suffering, and their management has major morbidity and cost consequences. High blood glucose concentrations not only cause acute metabolic problems but also lasting and accumulative damage by chemical reaction (glycation, e.g. Haemoglobin A1c) with a host of physiologically critical proteins. Hence the long term damage to the cardiovascular system, eye, kidney, heart and nervous system. On the other hand, low blood glucose levels, known as hypoglycaemic episodes, are usually perceived by the patient and treated by ingesting glucose or food. But in a significant minority of patients, hypoglycaemic episodes are not perceived and may cause loss of consciousness. These can lead to fatal outcomes for the patient and sometimes for others, such as in situations where the patient is in control of a moving vehicle.

For these reasons, the investment in, and search for, newer treatments that can provide a 'cure' for the disease with normoglycaemic control, or that at least minimizes the damaging effects of extremely high and low blood glucose excursions with markedly better control of metabolic disturbances, has been energetically pursued. In terms of health economics, the benefits in preventing eye, heart and kidney diseases would more than justify a substantial investment in such research and development (Beckwith et al. 2010).

### **1.1 Background and rationale for cell transplantation**

Insulin is essential for normal glucose metabolism. It is released by the beta-islet cells of the pancreas in response to rising blood glucose levels. The feedback mechanisms involved provide a precise, finely tuned response, keeping blood glucose at a concentration of around 4.5mM. Type 1 diabetes is an endocrine disorder caused by autoimmune destruction of the beta-islet cells leading to insulin deficiency. Treatment by injecting various commercially produced insulins subcutaneously, while life-sustaining, can not provide the control of blood glucose provided by a full complement of functional islets.

In order to optimize the control of blood sugar and thus prevent the acute and chronic damage, the most likely way to improve these outcomes is to replace the patient's pancreatic islets with a new pancreas or new islets. Transplanting a whole new pancreas is a very demanding procedure that requires many resources and is found to be less than practical.

#### **1.1.1 Porcine islet cell transplants**

Among the newer treatment strategies that have been proposed, transplantation of pancreatic islets, obtained either from other human or animal donors, has received considerable attention worldwide. This is because islet transplantation can restore not only the insulin-secreting unit, but also the precise insulin release in response to rising blood glucose and multiple signals arising within and beyond the islets.

Because human islet transplantation is limited by the shortage of human islet tissue, human embryonic stem cells or induced pluripotent stem (iPS) cells from the patient are being developed into transplantable insulin producing cells. Recent reports indicate that iPS cells may not be transplantable into mice of the same strain without immune rejection (Zhao et al, 2011). The US FDA guidelines highlight concerns that stem cell derived lines may undergo malignant transformation or develop into teratomas after transplantation (Fink et al 2009).

While stem cell line-derived insulin producing cells are still at an early stage of research, pig islets are viewed as a promising alternative since: (a) the supply of pig pancreatic cells can be

increased by breeding more donor animals; (b) pig and human insulins have close structural and biological similarities; and (c) physiological glucose levels in pigs are similar to those in humans (Elliott, 2011). The rationale for this treatment approach (termed 'xenotransplantation') is that the implanted pig islets have the potential to mimic the normal physiological insulin response in type 1 diabetics, such that near-normal blood glucose levels may be achievable without insulin injections or with reduced requirements. As a consequence, long-term diabetes complications may be prevented and patients should experience less hypoglycaemia than they do with the currently recommended 'intensive' insulin regimens. Thus the need and rationale for improved diabetes control is clear but the effectiveness and practicality of islet transplants, whether from human, porcine or other sources, has yet to be firmly established. As with any transplanted tissue, organ or cells, whether from human or animal, the host immune system must be considered. Immunosuppression has been studied and used extensively in islet transplant patients. An increasingly successful alternative is immune-isolation, that is to isolate the transplanted cells in capsules or devices that exclude immune cells and antibodies, but allow the free diffusion of glucose, insulin, nutrients and dissolved oxygen and carbon dioxide. The latter approach eliminates the risks associated with immunosuppression.

Some of the **key issues** in implementing **porcine islet xenotransplantation** in the treatment of patients with Type 1 diabetes are:

**1.1.1.1** Have 'proof of concept' pre-clinical experiments demonstrated sufficiently effective improvement in the control of Type 1 diabetes in experimental animals?

**1.1.1.2** Have the risks to the patient's safety been sufficiently evaluated, including: (a) the risk of transmission of infectious diseases; and (b) risks to the individual from the transplant procedure itself, including allergic reactions and other immunological responses that might compromise the success of the procedure.

**1.1.1.3** How rejection of the cells by the recipient's immune system can be effectively prevented.

**1.1.1.4** Whether porcine islet xenotransplants can restore, at least partially, the normal regulation of blood glucose (as reflected in decreased insulin requirements and decrease in HbA1c), and the number of islets needed to achieve this.

**1.1.1.5** The duration of effectiveness of the transplanted islets (i.e. whether they remain effective over a sufficiently prolonged period to justify the inconvenience and cost of the procedure), and the extent to which the patient's well-being is enhanced and long-term diabetes complications are prevented.

**1.1.1.6** Ethical considerations, including cultural, ethical and spiritual dimensions, informed consent issues, and measures to ensure animal welfare.

**1.1.1.7** If clinical trials are judged successful by rigorous independent review, will resources to expand the availability of the treatment be provided by commercial and/or government investment?

## **2. Pre-clinical studies of porcine islet xenotransplantation in non-human primates and rodents**

The main body of information from our laboratory on the efficacy and safety of current preparations of porcine islets is derived from studies with islets sourced from Auckland

Islands Pigs. The Auckland Islands (AI) strain is unique due to its isolation for more than 150 years on a sub-antarctic island and is free of pathogens commonly found in other pig herds. These animals are being bred in custom-constructed pathogen-free facilities for transplant purposes and are discussed in detail later in this chapter.

## 2.1 Studies in non-human primates

The first exploration of primate diabetes treatments with encapsulated porcine islets was undertaken by Sun Y et al (1996) in Toronto, Canada. This pioneering group prepared islets and enclosed them in alginate-polylysine capsules in the early 1980s (Sun AM et al, 1984; Sun Y et al, 1993). They were able to find a number of diabetic cynomolgus monkeys that were ideal for testing these encapsulated porcine islets. These animals responded very well to their encapsulated porcine islets transplant, with significant improvement of their diabetes for 120-800 days. This remarkable achievement was based on decades of research by AM Sun, Y Sun and their colleagues that began in the early 1970s.

In a later non-human primate study the clinical efficacy and safety of an encapsulated neonatal porcine islet preparation was investigated in cynomolgus monkeys with streptozotocin (STZ)-induced diabetes. The islet toxin streptozotocin is injected into the monkeys and causes insulin deficiency. Sixteen monkeys with the disease established were separated into two groups of 8: one group was given microcapsules containing living porcine islets and the other was given the same microcapsules but without islets in them. They received the microcapsule transplants in two doses three months apart by injection into the peritoneal cavity (Elliott et al, 2005). No immunosuppressive drugs were administered.

In the group that received capsules containing islets, evidence of clinical activity was noted at 12 and 24 weeks after the first transplant; the reduction of the mean weekly insulin requirement relative to the control was 36% ( $p=.02$ ) and 43% ( $p=.01$ ), respectively at these time points. Blood glucose in the two groups were maintained to similar levels indicating that the reduced requirement for insulin injections in the treated group was significant. Both the islet-treated and control groups tolerated the transplant procedures well. No hypoglycaemic episodes or other adverse events were observed in the islet-treated group. There were no differences between the two groups of monkeys in body weight or hematology and liver enzyme parameters. Two deaths occurred, one in the islet-treated group from lobar pneumonia with disseminated lung abscesses at 13 weeks after the first transplant (despite systemic antibiotic therapy), and the other in the control (empty microcapsules) group as a result of a stroke that followed the development of hypoglycaemia due to failure to consume food after regular insulin therapy.

At terminal autopsy of these monkeys (Figure 1), no gross inflammatory reactions to either encapsulated islets or the empty microcapsules were noted in the animals' peritoneal cavities. The organs (liver, spleen, stomach, intestines, kidney, heart, lungs and brain) appeared normal.

In another streptozotocin diabetic monkey study, where encapsulated adult porcine islets were used, and thus more similar to the adult human islet isolations, diabetes was successfully treated without immunosuppression (Dufrane et al, 2010). However, although efficacy was demonstrated with encapsulated islets, they lost their function after 2 weeks. Successful glucose control was achieved for 6 months using 'monolayer cellular devices' containing up to 30,000 Islet Equivalents (IEQ)/Kg of monkey weight, implanted in the abdominal subcutaneous tissue.



Fig. 1. Clusters of encapsulated porcine islets attached to the mesentery of a cynomolgus monkey.

In contrast, Hering et al (2006) demonstrated that unencapsulated naked porcine islets, injected into the portal vein of the liver, could be used to reverse diabetes for 100 days in cynomolgus monkeys provided comprehensive immunosuppression was given.

## 2.2 Studies in small animal models of diabetes

The biocompatibility of encapsulated porcine islets and a dose-dependent effect on glycaemic control have been demonstrated in STZ-diabetic rats. Those that developed diabetes were separated into four groups that received intraperitoneal transplants of encapsulated neonatal porcine islets at increasing dosage. The doses, measured in IEQs, were 3000 IEQ (n = 7), 6000 IEQ (n = 6), 12,000 IEQ (n = 6) or 18,000 IEQ (n = 6) of the standard preparation or empty alginate microcapsules (n = 12), insulin requirements were significantly reduced in rats given the higher doses. Prior to transplantation, all rats had comparable blood glucose control with daily isophane insulin injections. At week 12 after transplantation, the reduction in the weekly average daily insulin dose was significantly greater in rats given doses of 18,000 IEQs ( $p < 0.01$ ) or 12,000 IEQs ( $p < 0.05$ ) compared with control animals that received empty alginate microcapsules. Insulin independence was attained in 3 of 6 rats given 18,000 IEQs, 3 of 6 given 12,000 IEQs, 2 of 6 given 6000 IEQs. 1 of the 12 diabetic rats given empty alginate microcapsules did recover, a case of spontaneous islet regeneration which is a known confounding factor in small animal studies (Figure 2).

Similar encouraging results were seen when streptozotocin-induced diabetic mice and genetically predisposed diabetic NOD mice were treated with encapsulated islets.

Thus, immunosuppression may not be necessary when treating diabetes if the islets are efficiently sealed inside capsules or devices with semipermeable membranes. The capsule or device outer membranes exclude immune cells and immunoglobulins but must allow efficient diffusion of glucose, insulin, all nutrients and gases (oxygen,  $\text{CO}_2$ ) necessary for cell nutrition.

This information about the efficacy of encapsulated islets and their tolerance by animal recipients, including some earlier human clinical studies, were assembled in great detail for the submission of applications to the National Regulatory Clinical Trial Agencies of several countries including New Zealand, Russia and Argentina, where the applications were successful, albeit with stringent conditions attached.



Martin et al. 1998a) and that immune-incompetent SCID (severe combined immunodeficiency) mice may develop either microchimerism or infection *in vivo* (van der Laan et al. 2000). The *in vitro* findings have, however, been shown to be strain-specific (Patience 2001; Clemenceau et al. 2001; Oldmixon et al. 2002) and cells from animals studied by LCT have not shown retrovirus infectivity. Moreover, no evidence of PERV transmission has been detected over 200 patients who have been exposed to pig cells or tissues and tested for evidence of PERV infection using sensitive detection methods (Wynyard et al. 2011; Garkavenko et al. 2008b; Denner 2003; Dinsmore et al. 2000; Heneine et al. 1998; Heneine et al. 2001; Irgang et al. 2003; Martin et al. 1998b; Paradis et al. 1999; Patience et al. 1998; Tacke et al. 2001). In 2 New Zealand diabetic patients who received encapsulated porcine islet xenotransplants, no evidence of PERV proviral DNA or RNA was detectable in white blood cells and plasma up to 6 years after the transplant, and neither patient was found to have suffered any ill health as a result of the procedure (Elliott et al. 2000; Garkavenko et al. 2004a). Similarly, in 4 other patients who received unencapsulated islets in similar studies in NZ, no infection has been found in a follow-up time of up to 9 years (Garkavenko et al. 2004a). More recently one patient shown to have some functional transplanted porcine islets after 9.5 years was shown to be free of PERV.

### 3.2 Porcine endogenous retrovirus

In several other studies, no evidence of PERV transmission was found among recipients of porcine clotting factor VIII (Hyate:C) in a study of 88 haemophiliacs, despite the fact that all manufactured batches of porcine factor VIII concentrate used by patients were subsequently tested and shown to contain PERV RNA (Heneine et al. 2001). Similarly, no evidence of PERV DNA was found in 2 renal dialysis patients whose circulation had been linked extracorporeally to pig kidneys (Patience et al. 1998).

### 3.3 Auckland Islands pigs

A further refinement of the source herd has been obtained by the use of pigs derived from a colony abandoned on the Auckland Islands about 150 years ago. These animals are free of all measured viruses except the retrovirus (PERV), and are being bred for xenotransplant purposes in a purpose-built facility. Although PERV cannot be removed from the porcine genome, there is strong evidence that it is not functional in the Auckland Island Pig strain and is not expressed under any of the recommended testing conditions. This strain is now termed a "Null Pig Strain" suitable as a source of tissues for xenotransplantation (Wynyard et al, 2011).

### 3.4 Neonatal pigs

Nevertheless, the selection of donor animals that do not transmit infectious PERV continues to be important and has to be linked with the selection of donor animals bred in isolation and screened to exclude infection with other exogenous microbes. The use of donor neonatal piglets rather than adult pigs also has the advantage of limiting the exposure time of donor newborn animals to acquired infections as they age.

### 3.5 No genetic modification

The selection of piglets without genetic modification as a source of tissue offers another advantage. In attempting to prevent immune rejection of porcine tissue, genetically-modified



pigs with the gene for the xeno-antigen alpha-gal eliminated have been developed. However, with the use of alpha-gal gene knock-out pig donor cells, PERV-exiting cells are not enveloped (coated) with the alpha-gal antigen and hence are not recognised as 'foreign' by the recipient's blood complement system (Fujita et al. 2003). It is known that normal human serum contains natural anti-alpha-gal antibodies that inactivate retrovirus (Rother et al. 1995).

### **3.6 Intact immune system**

The maintenance of an intact immune system is an important safety factor. Immunosuppressive drugs are commonly used to prevent immune rejection of transplanted organs and cells. However, the use of alginate-encapsulated islets is intended to allow the survival of transplanted islets and their continued secretion of insulin in the recipient, without the need for life-long immunosuppressive drugs.

### **3.7 Surveillance**

Long-term surveillance of recipients of porcine islet xenotransplants and testing of the transplant material for the presence of PERVs, using highly specific and highly sensitive assays developed for this purpose will always be an integral part of strategies proposed for future clinical trials. This safeguard, which is a necessary precaution recommended for clinical trials of xenotransplantation by authorities in various countries including the USA (US Department of Health & Human Services 2002, 2003), Australia (National Health & Medical Research Council 2002), UK (United Kingdom Xenotransplantation Interim Regulatory Authority 1998, 1999), and Canada (Therapeutic Products Programme, Health Protection Branch, Health Canada 1999), is designed to allow early detection of infectious disease transmission, and includes standard hospital infection control measures to limit the spread of such an infection should one be detected. These carefully structured and independently monitored precautionary measures are now mandatory for all patients enrolled in clinical trials of porcine islet (or other cells) xenotransplantation and are put in place before the trials begin.

## **4. Minimizing risks of contaminating with infective agents during product preparation**

After the pancreas is removed from the donor animal it must be treated with a series of procedures to isolate the insulin-producing islets, substantially free of the proteolytic exocrine tissue which makes up the majority of the pancreas. It is then kept in cell culture medium and tested for microbiological contamination and for its ability to produce insulin in response to stimulation by exposure to a glucose challenge. This must be done with every batch of islets produced for clinical use. The manufacturing facility, records and staff training need to be regularly inspected by expert teams from government health agencies. Samples of islets from every batch and biopsies of pancreas, heart, lung, spleen, kidney and brain tissues, also blood samples, from every donor animal must also be stored frozen at -80 Centigrade as historical resources that can be retrospectively analyzed, in case of later complications in patients.

## **5. Preventing rejection of islets by the recipient's immune system**

The vulnerability of transplanted islets to the recipient's immune system has been a major scientific challenge and a barrier to successful islet transplantation. The transplanted cells

face not only Immediate Blood Mediated Immune Rejection (IBMIR) but also immune attack from a variety of cells which may result in loss of function and cell death. Two approaches are currently used to overcome this:

### 5.1 Immunosuppressive drugs

The administration of immunosuppressive drugs before a cell transplant and for the rest of the life of the patient. These procedures to manage the immune competence of the patient are essential in greater or lesser forms for all transplants of naked cells except perhaps those that are sourced from the patient (autotransplantation) or from a perfectly matched donor. Even then, any patient-derived cells (e.g. endogenous adult stem cells) that may be induced to mature into functional islet cells may be attacked by auto-immune processes engendered during the auto-immune destruction of the patient's original pancreatic islets, since the newly matured islet cells carry essentially the same antigens (Zhao et al, 2011).

A suppressed immune system, with lower surveillance of foreign antigens, can provide an opportunity for infection. Immunosuppressive agents may also prevent the essential immune surveillance that detects and destroys most cellular chance mutations that lead to cancer cell growth. This has been a serious concern about stem cell transplants that may contain a small number of viable undifferentiated cells that can exhibit uncontrolled replication and form tumours (Bauer SR, 2010).

### 5.2 Immunoisolation

Immunoprotection of the transplanted cells via the use of a semipermeable membrane that acts as a protective barrier (Figures 3a and 3b). The latter technique appears to be a viable approach, the principle being that the permeability of the outer capsule membrane allows smaller molecules such as glucose, dissolved oxygen and carbon dioxide and all nutrients to penetrate into the capsule and reach the islets. The membrane is constructed to allow insulin and most small proteins to be released out into the bloodstream, but it does not allow the passage of large immune cells or antibodies that would cause rejection of the islets. Indeed, the islets can survive well inside such systems. There are several approaches to providing this immunoprotection which are:

- Encapsulation of the islets within a bead of alginate gel, which is then coated with poly-L-ornithine, poly-L-lysine or some other material to provide perm-selectivity and strength (Calafiore 2006; Weir & Bonner-Weir 1997).
- Tubular diffusion chambers which consist of long (up to 20mm) tubular membranes of 5-6mm inner diameter.
- Perfusion devices (also known as vascularised artificial pancreases) which consist of an outer housing 90 mm in diameter and 20 mm in width that is connected surgically to the patient's vascular system so that it is fed by an artery and drained by a vein. Islets in both perfusion devices and diffusion chambers are usually immobilised with alginate or agar to prevent settling and to provide uniform distribution of nutrients and dissolved oxygen and carbob-dioxide (Maki et al. 1995).
- Co-transplantation with 'nursery' cells such as testicular Sertoli cells which have been claimed to protect against immune-mediated rejection via the production of the immunomodulator TGF-beta1 (transforming growth factor-beta 1) (Suarez-Pinzon et al. 2000; Valdes-Gonzalez, 2005).

Of these approaches, the strategy selected by Living Cell Technologies for intensive investigation is encapsulation of the islets in 'minimal volume' alginate microcapsules developed at the Department of Internal Medicine and Endocrine & Metabolic Services, University of Perugia in Italy (Calafiore 1997; Calafiore et al. 1999). Alginate-encapsulated porcine islets have been extensively investigated in experimental animals both with and without diabetes, and in a small number of human diabetic subjects.

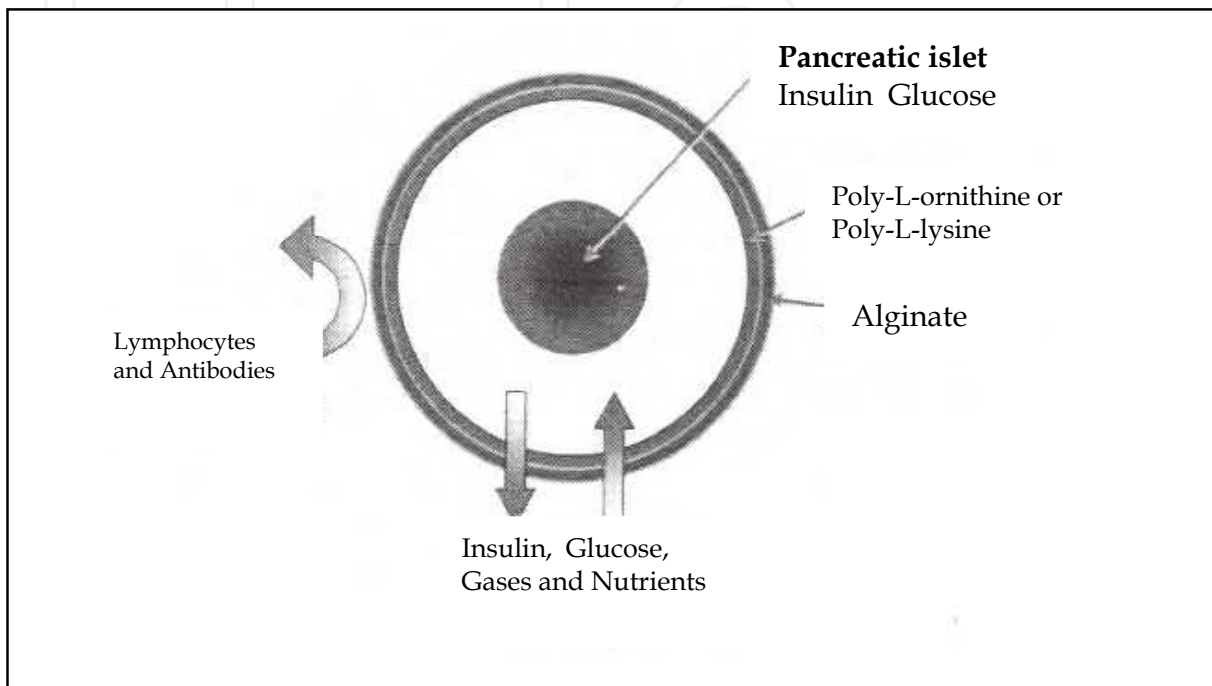


Fig. 3a. **The concept of encapsulation:** enclosure of the islet in an alginate/poly-L-ornithine or poly-L-lysine membrane that is permeable to glucose, nutrients and insulin, but not to lymphocytes and antibodies, provides protection against immune destruction of the cells.

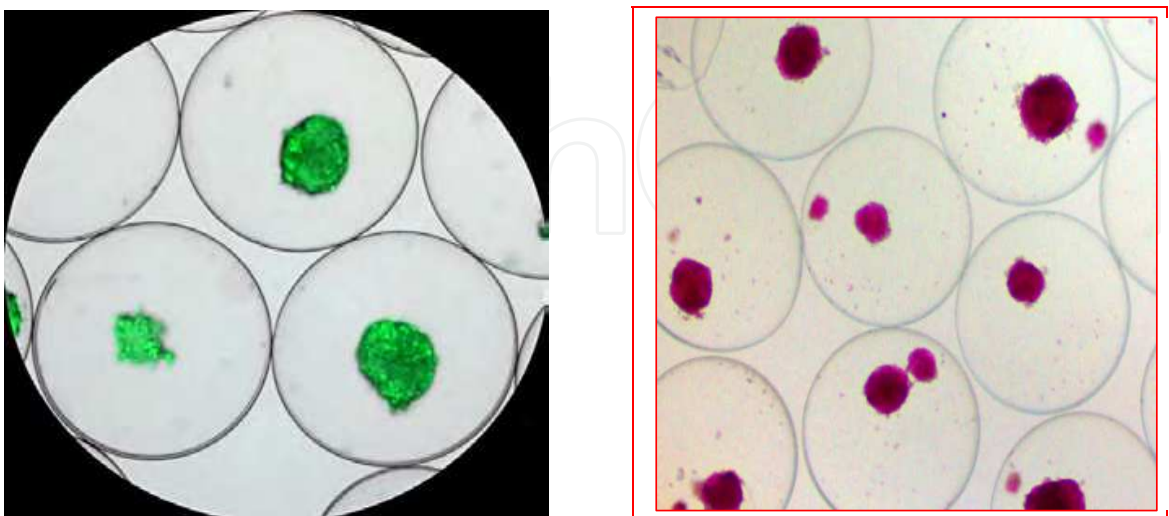


Fig. 3b. **Islets isolated from neonatal porcine pancreas in alginate-polyornithine capsules.** Green fluorescent stain (acridine orange) demonstrating viable cells and red tetra-methyl-rhodamine-ethyl ester (TMRE) stain for mitochondria of viable cells.

## 6. Alginate-poly-L-ornithine capsules

These capsules, as shown in **Figure 3**, have been the method of choice for many laboratories including our own (Opara & Kendall, 2002; Hernandez et al, 2010; Elliott, 2011). Their biocompatibility is critical to their *in vivo* efficacy and durability. Alginate is derived from seaweed and has to be highly purified to remove contaminating heavy metals, proteins and endotoxins. Different sources of alginate vary in their chemical composition with different ratios of their main constituents of mannuronic acid and guluronic acids and hence their physical gel properties. The consistency of the microcapsules with respect to their size, wall thickness, compressibility and cell occupancy has to be ensured from batch to batch. The central core containing the islets is liquefied alginate and the outer surface is a membrane formed by ionic interaction between positively charged poly-L-ornithine (or poly-L-lysine) and negatively charged alginate. Properly made, the membrane is remarkably robust (Skinner et al, 2009).

Since up to a million capsules may be required for treatment, the production must be reproducible, using durable equipment to Good Manufacturing Practice (GMP) standards. The main components are carefully engineered flow-through needles which generate reproducible sized droplets impelled by air 'knife' flows or electrostatic mechanisms (Hernandez et al, 2010).

## 7. Ethical considerations

Embodied in the wider concepts of informed consent are considerations that young people and the old or infirm are not exploited. Peoples with poor understanding of the language and scientific concepts involved are not to be misled. Religious and cultural feelings are not to be disregarded in potential recipients who find it difficult to express their reluctance to participate in the presence of those whom they perceive as medical authorities.

The ethical issues of xenotransplantation include concerns over the cultural, ethical and spiritual dimensions of xenotransplantation; the ethical acceptability of using animals to provide tissue for human transplantation; how the welfare of donor animals can be adequately protected and their suffering reduced; and how the welfare and interests of patients in early clinical xenotransplantation trials can be protected. These issues have been addressed by the Nuffield Council on Bioethics in the United Kingdom (1996) and, in New Zealand, the Bioethics Council, Toi te Taiao (2005). The latter body concluded that prohibition of xenotransplantation could not be justified, given the compelling human need argument, and that it should be allowed to develop in New Zealand, with that development being demonstrably shaped by the resolution and management of safety issues by a competent authority; the relationship between the majority European culture and indigenous Maori people and the cultural, ethical and spiritual factors that matter to most New Zealanders.

In recent years, a number of recommendations to protect the ethical integrity of future human research have been made by various regulatory authorities. Key issues in the conduct of clinical trials of xenotransplantation include the requirement to provide patients with an explanation of the likely success, its attendant risks, and the subsequent quality-of-life that can be expected when obtaining their informed consent, and informing patients that their consent to the procedure includes consent to ongoing post-transplantation microbiological monitoring.

Other international spiritual organisations have given their opinions about xenotransplantation. The Vatican in Rome, Italy have considered xenotransplantation in some depth and suggest it is worthy of serious consideration (Vatican, 2011). Jewish organisations have given qualified support to xenotransplantation as a life enhancing procedure while Muslim opinion is unclear but has generally been negative because of the status of pigs in the religious context.

## 8. Clinical experiences with xenotransplantation in treating type 1 diabetes

Many of the considerations in the preceding part of this chapter have been opinion and experiment seeking to determine the safety and efficacy of xenotransplantation for Type 1 diabetes in animal models. These animal models are perhaps more or less imperfect as true reflections of the human condition of Type 1 diabetes. It is therefore of critical relevance to review all data and experiences relating to the small number of human exposures to xenotransplanted porcine islets and other cells.

Six patients were treated in 1995-6 with either encapsulated or unencapsulated neonatal porcine islets. One of these, a 41-yr-old Caucasian male with type 1 diabetes for 18 years was given an intraperitoneal transplant of alginate-encapsulated porcine islets at the dose of 15,000 islet equivalents (IEQs)/kg bodyweight (total dose 1,305,000 IEQs) via laparoscopy. By 12 weeks following the transplant, his insulin dose was significantly reduced by 30% ( $p = 0.0001$ ). The insulin dose returned to the pre-transplant level at week 49. Improvement in glycaemic control continued as reflected by total glycated haemoglobin of 7.8% at 14 months from a pre-transplant level of 9.3%. Urinary porcine C-peptide, derived from the porcine pro-insulin precursor, peaked at 4 months (9.5 ng/ml) and remained detectable for 11 months (0.6 ng/ml). The patient was followed as part of a long-term microbiologic monitoring program which subsequently showed no evidence of porcine viral or retroviral infection.

The patient opted for elective laparoscopy 9.5 yr after transplantation. Abundant nodules were seen throughout the peritoneum. Biopsies of the nodules showed they contained capsules still protecting living cell clusters. 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 (Elliott et al, 2007).

With this demonstration it was clear that this form of xenotransplantation treatment has the potential for sustained benefit in human type 1 diabetics.

Since then two further human studies have started using porcine islets in microcapsules and without immunosuppressive drugs. In 2007, a pilot study with 8 patients was approved by the Scientific and Ethics Committees of the Sklifosovsky Institute, Moscow where islets were obtained from biocertified designated pathogen free pigs and encapsulated under GMP conditions in New Zealand. Adult patients were aged 23-63 with type 1 diabetes as defined by the American Diabetes Association criteria. They were insulin dependent for 5 -15 years. Before the implants, patients had to have stimulated plasma c-peptide levels  $< 0.2$  ng/ml to confirm insulin deficiency. Their diabetes had to be inadequately controlled with HbA1c of  $> 7\%$  pre-implant. Patients were administered 5,000 or 10,000 islet equivalents per kilogram body weight (IEQ/kg). There were no significant adverse events and no evidence of zoonosis. Patients were also given repeat implants with no untoward effects. Preliminary

data shows a reduction in daily insulin dose and reduction in HbA1c compared with pre-implant values following the first implant (Tables 1 and 2) in the majority of patients. Two patients became insulin independent for a period, the maximum being 32 weeks. At the repeat implant, 6 months after the first implant, intact microcapsules were retrieved and subsequently found to contain viable cells. Porcine insulin was also detected in the circulation following glucagon stimulation.

Patient ID	Dose 1st Tx (IEQ/kg)	Insulin Dose		% Dose Reduction	
		Pre 1st Tx	Post-1st Tx		
			3-month	6-month	
1	5,000	113	32	34	
2	5,000	22	100	32	
3	5,000	60	-7	5	
4	5,000	30	13	13	
5	5,000	68	15	16	
6	10,000	41	?	0	
7	10,000	37	73	100	
8	10,000	83	0	0	
Mean		57	32	25	

Table 1. Reduction in daily insulin dose after first implant (Tx) in 8-patient pilot study. Patient 2 was insulin-independent at 3 months but subsequently needed insulin support. Patient 7 became insulin-independent at 6 months.

Patient ID	Dose 1st Tx (IEQ/kg)	HbA1c (%)		
		Pre-1st Tx	Post-1st Tx	
			3-month	6-month
1	5,000	7.1	6.3	6.9
2	5,000	8.2	7.3	7.0
3	5,000	10.0	7.8	7.3
4	5,000	7.6	7.9	7.6
5	5,000	9.8	6.2	7.2
6	10,000	8.5	-	-
7	10,000	8.3	4.9	6.5
8	10,000	11.3	8.2	8.6
Mean:		8.9	6.9	7.3

Table 2. Reduction in Haemoglobin A1c (HbA1c) after first implant (Tx) in 8-patient pilot study.

In 2009, a Phase I/IIa study approved by the New Zealand government following international peer review and Ethics Committee Approval was commenced. It will include 14 adult patients. Unlike the preceding clinical studies, these patients were required to have unstable type 1 diabetes accompanied with hypoglycemic episodes. The patients are to be administered a single intra-abdominal implant of 5,000, 10,000, 15,000 or 20,000 IEQ/kg. To date no significant adverse events have been attributable to the treatment. At this early stage of the open label study, episodes of hypoglycemic unawareness (Figure 4) and hypoglycemic convulsions have been eliminated in the first patient. This was associated with significant reduction in the severity of hypoglycemic scores. A full one year follow-up of all patients is expected to be completed at the end of 2011. A third trial will be conducted in Argentina in 2011/2. The clinical studies are thus still at the stage of dose finding to determine the optimum dose and dosing regimen.

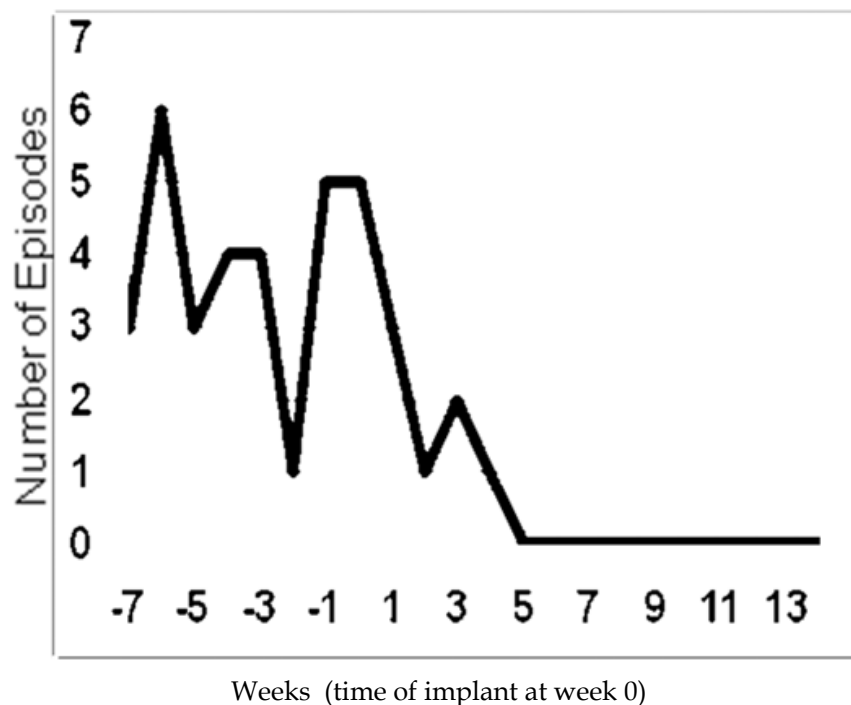


Fig. 4. Example of Elimination of Episodes of Unaware Hypoglycemia (Patient #1, Phase I/IIa Auckland, New Zealand)

## 9. Conclusion

Xenotransplantation is not yet standard treatment. There is current research into the feasibility of using donor animal organs such a kidney and liver. However, unlike cells, organs are difficult to screen against potential infectious agents. If clinical trials with cell transplants for diabetes are successful, porcine islet transplants will lead the way for the use of other cells for the treatment of nervous system disorders and enzyme deficiencies (Skinner et al, 2009).

Porcine islet xenotransplantation has the potential to be beneficial for those with absolute insulin deficiency. There is now a consensus that the procedure is relatively safe from xenotic infections. The results of several non-human primate and rodent studies indicate significant efficacy may be achieved. The early results of human clinical trials also suggest

that this form of treatment for Type 1 diabetes, without immunosuppression, is worthwhile. There is the concern that the treatment may be too expensive to be a practical treatment for the large numbers of patients who are expected to benefit from porcine islet implants. However, a health economic analysis of quality adjusted life years suggests that this approach may be cost effective taking into account the current cost of treating the disease and complications of cardiovascular disease, blindness, limb amputation, end-stage kidney disease and neuropathy (Beckwith et al, 2010).

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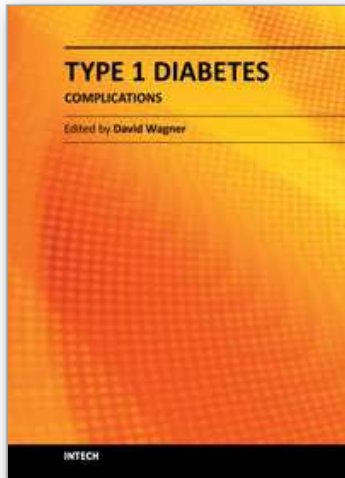
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