



Endogenous Insulin Delivery on Demand

Paul L J Tan, Kathleen Durbin and Robert Elliott of Living Cell Technologies Limited investigate porcine pancreatic islet implants without immunosuppression for insulin-dependent diabetes

The autoimmune destruction of pancreatic beta islet cells in type 1 diabetes severely disrupts normal glucose metabolism and causes a chronic and incurable life threatening disease. Treatment with exogenous insulin is life-saving, but requires a complicated daily regimen of multiple injections and blood glucose monitoring. Insulin dosing is adjusted to prevent fluctuations of blood glucose levels to limit episodes of hyperglycaemia. Failure to maintain euglycaemia results in micro- and macrovascular disease and the late complications of diabetes such as nephropathy, retinopathy, hypertension, diabetic ulcers, neuropathy and cardiomyopathy.

The American Diabetes Association recommends a target glycated haemoglobin level of less than seven per cent (HbA1c) as a reflection of consistent euglycemia such that haemoglobin is not abnormally glycated consequent to frequent or prolonged periods of hyperglycaemia. A normal HbA1c is a reliable surrogate predictor of reduced risk of the long term complications of diabetes (1).

While intensive insulin therapy has been encouraged primarily to avoid the delayed complications of diabetes, an intensive insulin regimen in many cases increases the occurrence of hypoglycaemic events (2). The Diabetes Control and Complications Trial reveals that most of the episodes of hypoglycaemia occur when the patient is asleep at night (3). Of great concern too is that, with chronic diabetes, patients frequently become unaware of impending hypoglycaemia, even when they are awake. New methods and devices for insulin delivery and monitoring continue to be developed to improve the management of blood glucose levels. Nevertheless, episodic hypoglycaemia and hypoglycaemic unawareness remain life-threatening risks for a significant number of patients with type 1 diabetes who follow medical advice to normalise their HbA1c level.

ENDOGENOUS INSULIN DELIVERY

An alternative approach to exogenous insulin injections is the replacement of lost beta cells with pancreas or pancreatic islet cells from human cadaver donors. Pancreatic transplantation has been successful, but requires immune suppressive drugs (4).

Furthermore, the allotransplantation programme is limited by the supply of human donor tissue.

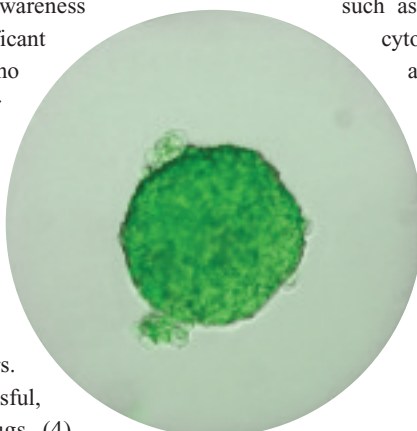
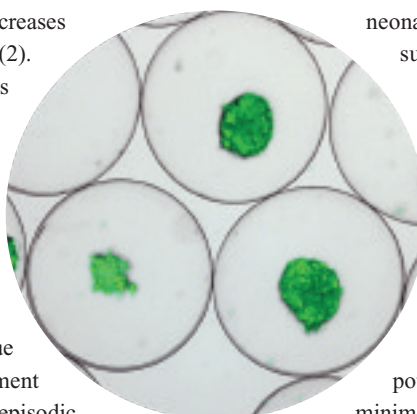
Human embryonic stem cells or induced pluripotent stem cells are potential sources of transplantable insulin-producing cells. In the US FDA guidelines for stem cell-based therapeutics, major concerns relate to the potential for teratoma to arise from such cells and for their spontaneous transformation into malignant cells (5).

Tissue from animal donors can provide a practical alternative to overcome the lack of human donor tissue. First attempted as long ago as 1994 in humans, the animal of choice is the pig, as porcine insulin is identical to human insulin except for one amino acid residue (6). Porcine insulin has been used for many decades to treat diabetes prior to the availability of recombinant human insulins. Porcine islets respond to glucose challenge by secreting insulin in a manner similar to human islets.

Adult, neonatal and foetal pancreatic tissue from pigs has been assessed as transplantable tissue in animal models.

Cells from islet preparations and pancreases from neonatal piglets have proliferative capability superior to the adult pancreas and contain ductal cells that are precursors for beta cell neogenesis (7). Whole foetal pancreata differentiate and mature *in vivo* into insulin-producing tissue following transplantation in recipient animals (8).

The use of animal tissue requires assurance that the risk of transmitting potential infections from the donor animal is minimised to an acceptable limit. Porcine viruses such as pig hepatitis E, circovirus type 2, pig cytomegalovirus, encephalomyocarditis virus, and pig lymphotropic herpesvirus are exogenous infectious agents in pigs which must be screened out (9). Unlike the exogenous viruses, porcine endogenous retroviruses (PERV) are present in the genome of all pigs. PERV is infectious for human cells *in vitro* though recent data from several laboratories indicate that PERV infection



Insulin producing cells in immunosolatory capsules

does not cross species *in vivo* (10,11). Nevertheless, the selected donor pigs should be confirmed as of a ‘null’ strain that does not secrete infectious PERV (12). Such pig herds have to be maintained in appropriate closed facilities to qualify as designated pathogen-free donors suitable as a source of tissue for human therapeutics.

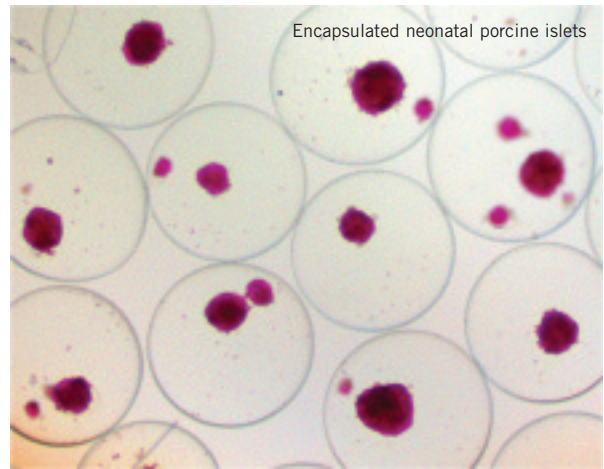
IMMUNE REJECTION

The transplantation of allogeneic or xenogeneic pancreatic islets has to overcome rejection by the host immune system. Immune suppressive drugs commonly used to prevent immune rejection confer a significant risk of drug adverse events. Immunosuppressive drugs are unavoidable in whole organ allo- and xenotransplantation, but cellular implants offer the attractive alternative of not suppressing but retaining a normal immune system. Islets may be implanted in an immunoisolatory material that is porous to permit the inward diffusion of oxygen, nutrients and glucose and the exit of insulin secreted in response to high ambient glucose concentration. This is achievable with the encapsulation of islets in alginate microspheres using material that is selected to be biocompatible. Significant advances have been made in recent years in the characterisation of encapsulation materials and the layer-by-layer fabrication of nanoporous microcapsules for islets to be implanted successfully to ameliorate diabetes in various animal models without the use of immunosuppressive drugs (13).

HUMAN STUDIES WITH PORCINE ISLETS

The long term follow-up after the implant of a prototype formulation of alginate encapsulated neonatal porcine islets into a patient with type 1 diabetes supports the notion that implanted islets may survive for long periods (14). In this specific case, porcine insulin was detectable in blood samples taken from the recipient following a glucose tolerance test done 10 years after the transplant. This observation is now to be investigated with another study approved by the New Zealand Government.

The approval under current regulatory guidelines is a consequence of a nationwide consultation by a government-appointed Bioethics Council, which in 2005 concluded that the populace was in favour of xenotransplantation being developed in New Zealand (15). Conditions that have to be met include the use of pigs that are ‘null’ and designated pathogen-free, that a molecular diagnostic laboratory be established and accredited as capable of screening donor animals against potential zoonoses, that the encapsulated porcine islets be prepared in a good manufacturing practice (GMP)-certified manufacturing unit, that a register of recipients, and an archive of biological samples be established so that recipients can be followed up long-term and that their blood samples be available for investigation in the event of unexplained infection or adverse event. The Minister of Health received recommendations from reviews by a Gene Technology and Advisory Committee and their international science referees, a National Health Committee,



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which sourced opinions from international referees and further submissions from the New Zealand public, a Regional Ethics Committee and an independent international expert reviewer.

CLINICAL TRIAL EXPECTATIONS

A clinical study of implants of cells that release endogenous insulin is an attractive opportunity for many patients with type 1 diabetes, and draws many volunteers. It is necessary to enrol patients who have the greatest potential to benefit, but who also appreciate the responsibility of rigorous monitoring and long-term follow-up after a xenogeneic cell implant. These and special considerations such as recipients and their life partners (or spouses) providing blood samples and having personal details on a national xenotransplantation register, potentially contravening personal privacy, have to be spelt out in the informed consent and patient enrolment process.

The clinical trial design is essentially an open-label study as it involves the surgical endoscopic placement of encapsulated cells (16). Although children with type 1 diabetes are the group most likely to benefit, first in human studies necessarily start with adults. The approved clinical trial protocol requires that the patient have poorly controlled or ‘brittle’ diabetes despite intensive insulin therapy supervised by a diabetologist.

Early clinical studies focus on the safety of the procedure and intervention. It is encouraging to note that cellular xenotransplants so far carried out have not encountered significant adverse events nor the transmission of potential zoonoses (17,18).

Early investigations suggest that encapsulated porcine islet implants function and secrete insulin in a regulated manner *in vivo* (14,19). The purpose of further clinical trials is to define the optimal dose, the magnitude and duration of benefit. The primary outcome is a normalised HbA1c with insulin independence or a significantly reduced dose of exogenous daily insulin. The most relevant benefit for patients would be the reduction or elimination of hypoglycaemic unawareness and a corresponding improvement in scores for a better quality of life.

It has taken a long time for the concept of using encapsulated porcine islets without immunosuppression to be developed for implantation into humans. Cell preparation and encapsulation technologies have made significant advances for regulatory bodies to sanction a clinical trial with current guidelines. The approval to proceed was based on the assurance that the pigs do not secrete infectious virus. The pigs are not transgenically humanised, hence if the implanted cells were to be released from damaged capsules, the implant may be promptly recognised by a competent host immune system that is not suppressed. In such a circumstance, the potential of zoonoses remains a theoretical risk, while the known and significant risk of immune suppression is eliminated. Diabetes patients more than anyone are looking to the possibility of an endogenous source of insulin on demand and a normal life which has so far evaded them.

References

- American Diabetes Association, Standards of medical care in diabetes – 2009, *Diabetes Care* 32: S13-61, 2009
- Sherwin RS, Bringing light to the dark side of insulin: A journey across the blood-brain barrier, *Diabetes* 57: pp2,259-2,268, 2008
- DCCT Research Group, The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent mellitus, *N Engl J Med* 329: pp977-986, 1993
- Shapiro AMJ *et al*, International trial of the Edmonton Protocol for islet transplantation, *N Engl J Med* 355: pp1,318-1,330, 2006
- Fink Jr DW, FDA regulation of stem cell-based products, *Science* 324: pp1,662-1,663, 2009
- Groth C and Korsgren O, Transplantation of porcine fetal pancreas to diabetic patients, *Lancet* 344: pp1,402-1,404, 1994
- Trivedi N *et al*, Increase in beta-cell mass in transplanted porcine neonatal pancreatic cell clusters is due to proliferation of beta-cells and differentiation of duct cells, *Endocrinology* 142: pp2,115-2,122, 2001
- Eventov-Friedman S *et al*, Embryonic pig pancreatic tissue transplantation for the treatment of diabetes, *PLoS Medicine* 3: pp1,165-1,177, 2006
- Garkavenko O *et al*, Porcine endogenous retrovirus transmission characteristics from a designated pathogen-free herd, *Transplant Proceedings* 12: pp209-216, 2005
- Yong-Guang Y *et al*, Mouse retrovirus mediates porcine endogenous retrovirus transmission into human cells in long-term human-porcine chimeric mice, *J Clin Invest* 114: pp695-700, 2004
- Specke V *et al*, No *in vivo* infection of triple immunosuppressed non-human primates after inoculation with high titres of porcine endogenous retroviruses, *Xenotransplantation* 16: pp34-44, 2009
- Garkavenko O *et al*, Porcine endogenous retrovirus (PERV) and its transmission characteristics: A study of the New Zealand designated pathogen-free herd, *Cell Transplant* 17: pp1,381-1,388, 2008
- Thanos CG and Elliott RB, Encapsulated porcine islet transplantation: an evolving therapy for the treatment of Type I diabetes, *Expert Opin Biol Ther* 9: pp29-44, 2009
- Elliott RB *et al*, Live encapsulated porcine islets from a type I diabetic patient 9.5 years after xenotransplantation, *Xenotransplantation* 14: pp157-161, 2007
- Bioethics Council, The cultural, ethical and spiritual aspects of animal-to-human transplantation: A report on xenotransplantation by toi te taiao: the bioethics council. Published in August 2005, www.bioethics.org.nz
- Living Cell Technologies, Open-label investigation of the safety and effectiveness of DIABECCELL® in patients with type 1 diabetes mellitus, www.clinicaltrials.gov
- Paradis K *et al*, Search for cross species transmission of porcine endogenous retrovirus in patients treated with living pig tissue, XEN III Study Group. *Science* 285: pp1,236-1,241, 1999
- Garkavenko O *et al*, Monitoring for presence of potentially xenotic viruses in recipients of pig islet xenotransplantation, *J Clin Microbiol*: 42: pp5,353-5,356, 2004
- Elliott RB *et al*, Intraperitoneal alginate-encapsulated neonatal porcine islets in a placebo-controlled study with 16 diabetic cynomolgus primates, *Transplantation Proceedings* 37: pp3,505-3,508, 2005

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