Comparison of bioartificial and artificial pancreatic transplantation as promising therapies for Type I Diabetes Mellitus

Katie Baker*

72 Leasowe Drive, Perton, Wolverhampton WV6 7TU, UK

*Corresponding author: 72 Leasowe Drive, Perton, Wolverhampton WV6 7TU, UK. Email: kbbaker@hotmail.co.uk

Supervisor: Dr David Watson, School of Life Sciences, Keele University, Keele, Staffordshire, ST5 5BG.

Type 1 diabetes mellitus (T1DM) is a chronic life-threatening condition whose incidence in the UK has doubled every 20 years since 1945 (Diabetes UK, 2010). Whilst intensive insulin therapy has been shown to reduce the incidence of long-term vascular complications in T1DM patients, it has also been shown to increase the risk of severe hypoglycaemia by 3-fold. Clinical islet transplantation has progressed considerably over the past decade, yet issues regarding the toxic effects of immunosuppression drugs and the paucity of pancreatic donor supplies remain. To provide an effective long-term therapy for heightened glycaemic control, many studies are investigating the potential of bioartificial islet encapsulation strategies and artificial bihormonal closed-loop systems. Following consideration of the basis of pancreatic transplantation, this article takes an in-depth look at both the benefits and limitations of bioartificial and artificial therapies and compares their potential in terms of providing an effective long-term solution to patients suffering with T1DM.

Key words: type 1 diabetes mellitus, pancreatic transplantation, islet encapsulation, bihormonal closed-loop system, glycaemic control, microcapsule device

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Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune condition resulting in β-cell apoptosis of the pancreatic islets of Langerhans, which develops due to an environmental trigger in genetically susceptible individuals (Knip and Simell, 2012). The primary soluble mediators of β cell loss are pro-inflammatory cytokines interleukin-1β, interferon-γ and tumour necrosis factor-α; secreted by activated mononuclear cells to induce differential expression of inflammatory response genes and pro-apoptotic hypoxia-inducible factor-α (Ortis et al., 2010).

Pancreatic β-cell function is crucial for glycaemia regulation; β cells act as physiological glucose sensors to detect elevated blood glucose (BG) levels during the absorptive state, stimulating insulin release for the promotion of glycolysis, glycogenesis and lipogenesis (Leibiger et al., 2008). When BG levels fall post-absorptive state, glucagon is released from pancreatic α cells, activating glycogen phosphorlyase to stimulate hepatic glycogenolysis and gluconeogenesis (Quesada et al., 2008). The level of homeostatic control achieved relies on the coordinated release of insulin and glucagon via negative feedback. Further hormone interactions involve somatostatin-secreting δ cells, which respond to post-prandial BG increase, acting to reduce gut motility and increase nutrient absorption. Approximately 1 million islets are distributed throughout the healthy adult human pancreas and each islet is then composed of ~2000 cells; 65–80% are β cells, 15–20% are α cells and <10% are δ cells (Quesada et al., 2008). This multicellular structure enables important paracrine interactions; β cells make significant contacts with other endocrine cells, necessary for appropriate insulin secretion.
Progressive loss of β cell mass by apoptosis, therefore, causes insulinopenia, leading to hyperglycaemia and ketoadisosis. The primary treatment for T1DM at present is the injection of exogenous insulin and regular monitoring of BG levels. Insulin injection has enabled successful management of diabetes since its first use in 1922 by Banting and Best; however, many patients develop chronic secondary complications involving renal insufficiency, neuropathological problems and impaired vision principally due to difficulty in maintaining long-term stable BG levels (Ichii and Ricordi, 2009).

The challenge of achieving tight BG control is exacerbated by ‘unaware’ hypoglycaemic episodes; whereby excessive circulating insulin and deficient glucagon response is met with impaired sympathoadrenal response, reducing warning symptoms and arousal from sleep (Hovorka et al., 2014). Recurrent episodes of untreated hypoglycaemia increase patient susceptibility to the development of hypoglycaemia-associated autonomic failure (HAAF) that can result in devastating immediate neurological consequences (Unger, 2012). Clinical studies have shown that hypoglycaemic unawareness can be reversed in just 2–3 weeks by complete avoidance of hypoglycaemic episodes (Unger, 2012), yet this requires improved therapies for continuous maintenance of safe BG levels, more effective than that provided by insulin injection alone.

**Pancreatic transplantation**

The first human whole-organ pancreatic transplantation for the treatment of T1DM was undertaken in 1966 by W.D. Kelly; this proved effective in restoring normoglycaemia and achieved insulin independence in >80% of patients beyond 1 year (Sutherland et al., 2001). Whole-organ pancreatic transplantation is now considered the standard therapy for T1DM patients with uncontrollable BG and end-stage renal failure; however, it retains the risks associated with major surgical procedures and has possible complications related to exocrine tissue enzyme production (Demartines et al., 2005). High mortality and low islet graft survival rate chiefly explains the limited application of pancreatic transplantation. Technical failure rates in surgical procedure of pancreatic transplant have now decreased to ~8%; however, vascular graft thrombosis, intra-abdominal infection and reperfusion pancreatitis remain issues in patient and graft survival (Troppmann, 2010). Furthermore, according to ‘NHS Blood and Transplant’ for routine whole-organ pancreatic transplantation, the waiting time is between 1 and 2 years, calculated individually based on numerous factors including total HLA mismatch, sensitization and dialysis status.

As islets represent <2% of the pancreas, experimentation was carried out to determine the feasibility of transplanting islets alone. In the year 2000, seven patients with T1DM received islets from multiple donors during a study known as the Edmonton Protocol. The study showed the successful use of steroid-free immunosuppression and transplantation of larger islet mass for the restoration of long-term endogenous insulin production and glycaemic stability, protecting patients from severe hypoglycaemia even when insulin independence was not sustained (Shapiro et al., 2006). Patients now considered for islet transplantation include those who are C-peptide negative and experiencing recurrent hypoglycaemic episodes. This is primarily because the adverse effects of life-long chronic immunosuppression can be justified with the risk otherwise posed by hypoglycaemic unawareness.

**Limitations associated with islet transplantation**

Whilst islet allotransplantation has shown promise towards a more effective treatment than daily insulin injections, it is currently performed in small numbers. This can be explained partly by the requirement for chronic immunosuppressive medication against inflammatory cytokines, enabling opportunistic infections such as pneumonia and herpes to invade the host, and often causing significant adverse effects including anaemia, leukopenia, diarrhoea, vomiting and fatigue (Shapiro et al., 2006). According to some studies, immunosuppressants such as tacrolimus and sirolimus may also exhibit islet toxicity; impairing islet viability and graft function (Drachenberg et al., 1999).

Another major limiting factor in islet allotransplantation is the profound scarcity of healthy human donor organs (Buder et al., 2013). Over 11,000 islet equivalents per kilogram (IEQ/kg) of body weight are recommended per transplant for effective graft function, requiring 3 or 4 pancreases due to difficulty in separating islets from surrounding exocrine tissue (Sakata et al., 2012). Therefore, coupled to donor shortage is the equally significant issue of poor islet isolation efficiency and low islet cell yield, limiting attainment of appropriate mass required for transplantation. The Edmonton Protocol is typically followed as the standard procedure for isolation and islet culture pre-transplant; key elements include procurement of donor pancreas via controlled ductal perfusion, organ preservation in Winconsin solution, pancreas digestion using the Ricordi system and islet purification via continuous density gradient. Islet-cell product must then be transplanted percutaneously into the transhepatic portal vein within 3–4 h of isolation by simply gravity infusion; most commonly, the use of at least two fresh islet infusions are required with expected purity of >30% (Shapiro et al., 2006). More recently, studies have suggested the use of an additional purification step (rescue density gradient) to improve recovery of trapped islets (Miki et al., 2013).

Further loss of the transplanted islet mass (up to 70%) takes place immediately post-transplant due to an instant blood-mediated inflammatory response (BMIR). During an BMIR, platelets bind rapidly to the islet surface, contributing to the formation of a continuous fibrin capsule surrounding both islets and infiltrating polymorphonuclear leucocytes. This causes disruption of normal islet morphology, whilst activating the complement cascade which mediates βcell lysis (Ozmen et al., 2002). Studies have demonstrated the protective effect of a potent caspase inhibitor, IDN-6556 in rodent
hepatic and swine intraportal islet autotransplantation, with markedly reduced apoptotic activity suggesting potential for reducing islet loss (Hoglen et al., 2007; McCall et al., 2012).

Overall, the rate of insulin independence in patients following islet transplantation is known to diminish over time, with ~55% remaining insulin-free after 2 years and only ~15% after 5 years (McCall et al., 2012). In order to better this prognosis, the development of bioartificial and artificial pancreatic transplantation has more recently shown great promise towards heightened BG control. A comparison of these alternative strategies will be discussed to evaluate their success in overcoming the limitations associated with islet allotransplantation and to consider their potential for therapeutic intervention in T1DM.

**Islet encapsulation strategies**

Islet encapsulation also referred to as a ‘bioartificial’ pancreas, involves the envelopment of isolated islets in high polymer material, in order to provide a selectively permeable barrier to protect inner islets from mechanical stress and the host immune system. Meanwhile, this allows the bidirectional diffusion of nutrients, oxygen and waste and glucose-responsive release of insulin (Sakata et al., 2012).

**Comparison of different types of encapsulation**

There are three major approaches to islet encapsulation including intravascular macroencapsulation, and extravascular macro- and microencapsulation. Intravascular devices are anastomosed to the vascular system, placed within a hollow semi-permeable membrane separating islet clusters from the host systemic circulation. However, thrombus formation within the device lumen along with haemorrhage and infection has forced intravascular devices to be largely abandoned (Vos and Marchetti, 2002).

In 1916, Frederick Charles Pybus attempted the first allotransplantation of pancreatic tissue; grafting cadaveric pancreatic fragments into the abdomen of two T1DM patients without therapeutic success. Pybus understood that although transplantation was the rationale treatment, until the principles of grafting were established their attempts would continue to fail (Pybus, 1924). Previously effective transplantation sites were not exploited; currently islet transplants are delivered intraportally; however, when considering encapsulation, the increased islet diameter and demand to accommodate larger islet volume requires alternative transplant sites to prevent blockage of small hepatic vessels and ducts.

In extravascular macrocapsules, islets are immunoisolated within membrane-diffusion chambers implanted with minimal surgery in the peritoneal cavity, the subcutaneous site and the renal capsule and easily removal when infected. Due to their shape, macrocapsules made of polysulphone hollow fibres have a tendency to bend under physiological stress and therefore tubes with wider lumens have been used (Vos et al., 2002). However, the use of wider lumens increases the diffusion distance and decreases permeability of the macrocapsule in comparison to microcapsules, causing insufficient nutrient supply and waste material accumulation. Initial studies on islet macroencapsulation were unsuccessful due to aggregation of the grafted tissue allowing extensive necrosis in the centre of islet clusters (Lacy et al., 1991). This was solved by preventing contact between the tissue elements through permanent immobilization in a matrix such as collagen, chitosan or alginate (Vos et al., 2002).

During microencapsulation, each islet is enveloped in its own spherical semi-permeable membrane ranging from 100 nm to 1 mm in size, with generally one or a few islets per capsule as shown in Fig. 1. This offers greater diffusion capacity and a larger surface area enabling rapid response to BG fluctuation. In extravascular microencapsulation, islets are implanted in the peritoneal cavity in a prevascularized solid-support system for an optimal exchange of insulin and glucose and improved islet nutrition (Vos and Marchetti, 2002).

Alginate was the first material to be developed for use in microencapsulation and now remains the favoured material of choice due to its bioneutral nature, good stability and resistance to oxidative damage, and relatively low cost (Lee and Mooney, 2012). Alginate is a naturally occurring anionic polymer typically obtained from brown algae (Phaeophyceae) and consists of unbranched binary copolymers of β-(1-4)-d-mannuronic acid and α-(1-4)-l-guluronic acid of varying composition (Sakata et al., 2012). Moreover, the addition of materials such as polyethylene glycol and poly-l-lysine have been shown to reduce plasma absorption and increase membrane stability to block the diffusion of serum immunoglobulin, albumin and haemoglobin in vitro (Desai et al., 2000).

A significant issue in encapsulation is total graft volume; the average islet diameter is 150 μM with the surrounding capsule increasing their size 3-fold (Dufrane and Gianello, 2012). In clinical settings, viable transplant sites are unable to naturally carry the required volume of >100 ml of capsules. To increase islet concentration relative to implant volume, alginate beads are coated with a very thin membrane of poly-amino acids providing capsules of ~200 μm while maintaining a liquid core structure. Microcapsules can be implanted into the patient by a simple injection procedure and are not easily disrupted; however, they are more difficult to remove completely when necessary in comparison to macrocapsules (Vos et al., 2002).

One of the first in vivo studies performed intraperitoneal allotransplantation of alginate–polyslyne microencapsulated and unencapsulated islets in rats with streptozotocin-induced diabetes without immunosuppression. Post-transplant the encapsulated islets survived 3 weeks and remained morphologically intact over 15 weeks, whilst unencapsulated islets survived only 8 days (Lim and Sun, 1980). By 1993, microencapsulated islet allografts injected intraperitoneally in...
spontaneous diabetic canine studies were found to survive up to 726 days evidenced by positive C-peptide release (Soon-Shiong et al., 1993). Following promising results in large-animal studies, the first human clinical trial was performed in a 38-year-old male with T1DM. Cadaveric human islets encapsulated in alginate microcapsules were placed intraperitoneal at 10 000 IEQ/kg with 5000 IEQ/kg booster given 6 months later. The patient was able to discontinue all exogenous insulin at 9 months, however was on anti-rejection mediation due to renal transplantation (Soon-Shiong et al., 1994). After 25 years of intense pre-clinical study on microencapsulated islet allografts, phase 1 pilot human clinical trials with four patients have now been completed and followed-up 5 years post-transplant (Calafiore and Basta, 2014). Poly-l-ornithine-coated ultrapure alginate microcapsules were grafted intraperitoneally into non-immunosuppressed T1DM patients, giving a decrease in daily insulin consumption, HbA1c levels and hypoglycaemic unawareness. Graft function was confirmed by detectable C-peptide levels in all patients, and the absence of host immune sensitization reflected long-term safety of the bioconstruct. Calafiore and Basta placed emphasis on the importance of the composition of alginate encapsulation material.

Nanoencapsulation has also been investigated to minimize transplant volume, using a layer-by-layer assembly technique where islet surfaces are coated with reactive-polymer segments such as the insulinotropic ligand, GLP-1, to encourage increased glucose-responsive insulin secretion (Kizilel et al., 2010). Furthermore, nanoencapsulation is intended to alleviate post-transplant IBMIR; diabetic mouse models have shown that nanoencapsulated islets with phosphorylcholine-modified polysaccharide coatings can significantly extend survival of transplanted islets (Zhi et al., 2013). Nanoencapsulation is far from clinical application; however, research suggests its potential in increasing graft survival in vivo.
**Alternative supply of donor islets**

The bio-protection provided by encapsulation against immunological responses enabled investigation into the use of xenogeneic porcine islets. Initial human studies transplanted microencapsulated neonatal porcine islets combined with serial tol cells subcutaneously into the abdomen wall of 12 non-immunosuppressed T1DM patients. No porcine endogenous retrovirus (PERV) infection was detected nor did any significant adverse effects manifest, half of the patients showed a significant reduction in exogenous insulin requirements compared to pre-transplant levels, and glucose-stimulated porcine insulin was detected in the sera of three patients up to 4 years post-transplant (Valdes-Gonzalez et al., 2005). Unfortunately, a 7-year follow-up showed that all patients had returned back to their pre-transplant insulin doses (Valdes-Gonzalez et al., 2010).

Furthermore, in one study, a diabetic male was given an intraperitoneal xenotransplant of allogene-cell-encapsulated porcine islets at 15 000 IEQ/kg dosage. The patients HbA1c levels decreased from 9.3 to 7.8% within 14 months of receiving the transplant and produced detectable C-peptide for up to 11 months (0.6 ng/ml). However, 49 weeks post-transplant, their insulin requirements returned to pre-transplant levels. In order to understand why treatment slowly ceases to work, laparoscopy analysis was done 9.5 years post-transplantation. This showed abundant nodules throughout the peritoneum, containing opacified capsules with moderate insulin and glucagon staining cells (Elliott et al., 2007). Porcine xenotransplantation is a promising approach to overcoming the shortage of human donor without the need for toxic anti-rejection therapy; however, existing technology must improve in order for therapy to be effective long-term.

The more recent development of cell-based therapies is also intended to alleviate issues of donor supply, exploiting the promise offered by human stem cells produced in vitro for endogenous insulin production. In 2010 Alipio et al. demonstrated the reversal of hyperglycaemia in vivo using induced pluripotent stem cells (iPSC) differentiated into mature β-like cells. Transplantation of iPSC in non-obese diabetic mice resulted in kidney engraftment and sufficient normalization of BG detected via insulin secretion (Jeon et al., 2012). However, attempts for insulin-producing cells gave only β-cell like forms, lacking many functional characteristics and often of polyhormonal nature resembling transient endocrine cells (Bruina et al., 2014). By October 2014, Douglas Melton’s group at Harvard reported the successful in vitro generation of functional human stem cell-β (SC-β) cells from human iPSC. Transplantation of 5 million SC-β cells under the kidney capsule of immunocompromised mice revealed that human embryonic cells and iPSC package and secrete insulin into the host bloodstream within 2 weeks post-transplantation in glycemic-regulatory manner (Pagliuca et al., 2014). iPSC can be generated from embryonic fibroblasts or pancreas-derived epithelial cells from the ultimate recipient of the transplant and are therefore not faced with allo-rejection (Bruns et al., 2013). The results described suggest that SC-β cells present an opportunity for therapeutic development, yet human clinical trials are not likely to start for several years. Moreover, factors contributing to cell loss post-transplant remain including IBMR and therefore encapsulation strategies in combination would be essential.

Another limitation faced in islet transplantation and cell-based therapies recognizes that β cells require extensive intra-islet communication in vivo (Bavamian et al., 2007). The expression of connexion-36 protein forms permselective channels that permit diffusion of cytosolic molecules between adjacent β cells within individual islets, to significantly regulate biosynthesis, storage and release of insulin (Bavamian et al., 2007). The cell surface adhesion protein epithelial (E)–cadherin (ECAD) also plays an essential role in allowing β cells to cluster into islet structures (Rogers et al., 2007). This has been shown in mouse β cells whereby the down-regulation of ECAD contributes to abnormal islet architecture and reduced insulin secretion (Yamagata et al., 2002). Following understanding of the importance of synchronous and cooperative activity of intact islets, the need to engineer IPS cell-derived β cells into ‘pseudo-islet’ clusters (including other endocrine cell types) is paramount, to allow a coordinated regulatory network for fine-tuned effective insulin secretion and BG stability (Hoang et al., 2014).

**Issues with fibrosis and hypoxia**

Microcapsules are designed to be immunogenically bioinvisible, yet diffusion of low-molecular-weight cytotoxic molecules due to incomplete encapsulation can successfully induce an instant blood-mediated inflammatory reaction (Su et al., 2010). This leads to progressive thickening and scarring of fibroproliferative connective tissue, which inhibits blood supply causing severe hypoxia and β-cell apoptosis (Weir, 2013). To ensure complete encapsulation, the polyionic charge of islet surfaces can now be exploited as a binding site for polyionic coatings (Krist et al., 2006).

The ideal PO2 of peritoneal fluid surrounding islets for optimal function has been determined at 60 mmHg, with islet function at 50% by 27 mmHg and only 2% by 5 mmHg (Dionne et al., 1993). One approach to resolve hypoxia is to engineer an oxygen-generating biomaterial. In 2012, Pedraza et al. used solid CaO2 in polydimethylsiloxane to deliver oxygen to the encapsulated islets for 6 weeks, demonstrating improved islet function via increased insulin release in vitro. Studies have also shown how the temporary release of co-encapsulated steroids such as dexamethasone can decrease ED-1/2-positive macrophages and neutrophil-mediated inflammatory responses resulting in less fibrosis than those transplanted without (Bunger et al., 2005).

The bioengineering of a durable ‘bioartificial’ pancreas hopes to improve long-term functionality of islets, whilst eliminating the need for toxic immunosuppressant drugs. However, many issues that limit its wider clinical application are still to be resolved including insufficient donor islet supply and total graft volume, fibrotic formation and islet hypoxia.
Alternative studies have shifted their focus to improving methods of BG control through enhanced exogenous delivery of insulin with the development of an ‘artificial’ pancreas. In 1974, Albisser et al. were among the first to describe an artificial pancreas as a computerized control system. The system relied on the administration of glucose or insulin with a high level of complexity and could therefore only be used within carefully supervised inpatient settings. Recent advances in the accuracy and performance of the latest generation of closed-loop system components have accelerated the development of devices, ultimately intended for outpatient use (Peyser et al., 2014).

The modern artificial pancreas is composed of three major functional components: a continuous glucose-monitoring (CGM) system, an insulin-infusion pump and a control algorithm (Fig. 2). The CGM is inserted subcutaneously at a depth of 8–12 mm in order to measure glucose in the interstitial fluid rather than intravascular; recent studies have shown that the physiological lag time between interstitial fluid and intravascular glucose is only 5–6 min (Basu et al., 2013). Insulin-infusion pumps are small, reliable electromechanical devices that provide programmed injections of insulin and glucagon into the subcutaneous tissue. These pumps consist of refillable cartridges and have a user interface enabling patients to establish a basal infusion rate and to give discrete bolus for coverage of a meal or correction of hyperglycaemia (Peyser et al., 2014). Perhaps, the most important component of an artificial pancreas is the control algorithms incorporated into a microprocessor device, which automatically calculate real-time insulin or glucagon (bihormonal) dosage based on data input from the CGM and insulin pump, to achieve specified target BG concentration. The three main algorithms include model predictive control (MPC), which anticipates future glucose trends in insulin administration, proportional integral-derivative (PID) control, which continuously adjusts insulin infusion rates, and fuzzy logic, which takes into account patient characteristics and basal/bolus factors (Shah et al., 2014).

**Unihormonal vs. bihormonal systems**

There are two major level approaches to achieving closed-loop BG control: unihormonal artificial pancreas systems infuse only insulin to reduce BG concentration, whilst bihormonal systems deliver insulin during hyperglycaemia and glucagon during hypoglycaemia (Peyser et al., 2014). Castle et al. (2010)
studied 14 T1DM patients undergoing closed-loop therapy with unihormonal or bihormonal control. In comparison to unihormonal delivery, results showed that bihormonal delivery significantly reduced the time spent in the hypoglycaemic range (15 ± 6 vs. 40 ± 10 min/day, p = 0.04) and significantly reduced the need for carbohydrate treatment (1.4 ± 0.8 vs. 4 ± 1.4 treatments/day, p = 0.01). It was concluded that high-gain pulses of glucagon improved glycaemic control with little risk of hypoglycaemia; however, long-term studies are necessary to assess the effect of ongoing glucagon treatment.

In order to compare bihormonal and unihormonal closed-loop systems in a more systematized method, Gao et al. (2013) used computer simulation in silico testing. Four types of closed-loop control methods were compared on 10 virtual hypoglycaemic subjects: insulin-only therapy, prediction insulin-suspending therapy and insulin-glucagon dual-infusion therapy including proportional (P-type) and proportional-derivative (PD-type) switching rules. The subjects under switching bihormonal systems maintained significantly lower BG indexes at 0.7 and 0.55 for P-type and PD-type, respectively, in comparison to 3.43 and 3.13 for insulin-only therapy and prediction insulin-suspending therapy, respectively (Gao et al., 2013). Bihormonal systems also appeared to prevent hypoglycaemia (BG < 70 mg/dl) and were found to be extremely robust in respect to hormone sensitivity variations, measurement noises and intersubject variability (Gao et al., 2013). Overall, the results suggested the superiority of PD-type switching rule maintaining stable BG levels (Gao et al., 2013).

MPC algorithms ‘control to zone’ design has recently been reported in several publications, where the objective is to bring glycaemic levels into an acceptable zone or range, rather than a strict and artificial target (Gondhalekar et al., 2013). Furthermore, eventually multiparametric MPC algorithms may be adapted for personalized models available to each individual subject (Dassau et al., 2013).

Moreover, numerous clinical trials have shown that the amylin analogue pramlintide acetate significantly reduces postprandial hyperglycaemia by slowing gastric emptying, and so can decrease insulin requirements by 30–50% (Ratner et al., 2005). Weinzierler et al. (2012) studied eight T1DM subjects using a closed-loop system with an insulin feedback algorithm; allowing 24 h on closed-loop control alone and 24 h on closed-loop plus 30 µg pre-meal pramlintide injection. The use of pramlintide significantly delayed time-to-peak BG and significantly reduced glycaemic excursion from target 120 mg/dl compared with control. It has been widely suggested that in order to provide further improvement in glycaemic control, the bihormonal approach may be expanded to include pramlintide (Micheletto et al., 2013).

Pharmokinetics in clinical studies

Clinical studies carried out by El-Khatib et al. in 2010 followed up 11 subjects with T1DM, the effect of bihormonal closed-loop systems was investigated for 27 h using the fast-acting insulin analogue lispro and glucagon. Results showed that six subjects achieved a mean BG concentration of 140 mg/dl with no instances of hypoglycaemia, whilst five subjects did exhibit hypoglycaemia requiring carbohydrate intervention due to slower lispro absorption (mean time-to-peak 117 ± 48 vs. 64 ± 4 min). Following preliminary results, adjustment of pharmokinetic (PK) parameters prevented hypoglycaemia in all subjects, whilst still achieving a mean BG concentration of 164 mg/dl (El-Khatib et al., 2010). The use of a customized MPC algorithm provided heightened glycaemic control, incorporating both subject weight and a PK model of subcutaneous absorption and clearance of lispro from the blood. However, the algorithm could not anticipate insulin absorption for individuals with slower lispro PK so commanded further insulin doses, leading to excessive plasma insulin in the late postprandial stage resulting in hypoglycaemia. This explains intersubject variability in closed-loop system performance (El-Khatib et al., 2010). The use of model-based predictive algorithms for both insulin and glucagon can permit dose instructions for future predicted glucose values and modification if secretion is inadequate; this produces a larger margin of safety for the prevention of hypoglycaemia (Peyser et al., 2014).

More recently, Hovorka et al. (2014) evaluated overnight closed-loop insulin delivery in 16 young people with T1DM in a free-living randomized clinical trial. Participants underwent two 21-day periods of sensor-augmented pump therapy with and without overnight closed-loop. Every 12 min, the treat-to-target algorithm calculated insulin-infusion rate using a compartment model of glucose kinetics, accounting for patient weight, total daily insulin dose and carbohydrate content of meals. The closed-loop system significantly increased the time of glucose in target range during both day and night with fewer episodes of nocturnal hypoglycaemia. Compared with control conditions, closed-loop therapy reduced mean overnight glucose by a mean of 14 mg/dl and almost halved the number of nights when glucose was <63 mg/dl for at least 20 min (Hovorka et al., 2014).

Closed-loop studies have revealed substantial night-to-night variability in insulin requirements, with the amount ranging between 50 and 200% from that given during control (Hovorka et al., 2014). Difficulty in achieving consistent nocturnal glucose levels with insulin pump therapy alone confirms the potential for closed-loop approaches. Results indicate that integration of closed-loop therapy into a normal living routine with varied diet and sleeping patterns is feasible, and conclude that unsupervised home-use of overnight closed-loop systems is safe (Hovorka et al., 2014).

Obstacles to overcome

Despite promising results, there are no large-scale clinical trials on fully implanted bihormonal closed-loop systems at the present time. This is because a number of challenges must be solved before the successful development of commercially viable artificial pancreas devices. Firstly the functional components of
the artificial pancreas must be maintained; sensors of the CGM must be replaced every 7 days and require frequency calibration (Shah et al., 2014), the device must be recharged regularly and insulin pumps refilled every 3 days (Brown and Edelman, 2010). The insertion of CGM subcutaneously is an invasive procedure and thus results in an inflammatory response at the insertion site that may produce inaccurate CGM data (Shah et al., 2014) whilst catheter blockage may prevent insulin delivery. Secondly, changes in insulin sensitivity can occur based on concurrent illness, unusual levels of physical activity and medication taken by the patient.

Improved formulations of insulin and glucagon are required before bihormonal closed-loop systems can enter an outpatient setting. The time from subcutaneous delivery to peak action of rapid-acting insulin analogues is around 90 min; in addition to this, the individual variability of absorption and clearance of insulin analogues makes it increasingly difficult to imitate normal human physiological conditions where insulin action is immediate (Shah et al., 2014). A review by Cengiz (2013) discusses the different approaches for producing insulin with more favourable pharmacokinetics to facilitate quicker treatment of hyperglycaemia, including localized heating, inhaled insulins and interperitoneal delivery. Glucagon is chemically and physically unstable and therefore a new solution must be prepared every 8 h; it is also sparingly soluble and has a tendency to fibrillate in solution which can induce an immunogenic response in patients (Brown and Edelman, 2010).

Another significant limitation is the suboptimal accuracy and reliability of commercially available CGM systems, which can give a relative absolute difference between sensor and reference glucose measurements of up to 15% (Thabit and Hovorka, 2012). These deviations often relate to sensor overestimation or mechanical perturbation; erroneous calibration or inappropriate algorithms (Thabit and Hovorka, 2012). Such a persistent deviation may cause insulin over-delivery and so increased risk of hypoglycaemia, posing the greatest challenge to closed-loop insulin delivery. Additional technical problems include the challenge of integrating CGMs, insulin pumps and system algorithms from different commercial entities, and there is also not currently a single insulin pump with PK customised algorithms but are behind in terms of development due to a number of limitations. These include the invasive procedure of device transplantation causing inflammation that gives inaccurate CGM data, the requirement for frequent maintenance and changing of system components, the inadequate formulations of insulin and glucagon and the suboptimal accuracy and reliability of CGM with transient deviations including sensor overestimation.

Recent work in human stem cell systems is promising; however, a major goal will be using these cells to produce pseudo-islets capable of the same level of bihormonal control offered in islet systems. Conclusively bioartificial encapsulation is more effective in maintaining normoglycaemia compared with closed-loop bihormonal artificial systems and is more widely accepted as a natural, safer therapy. However, the scarcity of donor supplies and the long-term effectiveness of this approach remain significant challenges. Following the positive outcomes in terms of capsule material qualification, solving these two prominent issues should result in islet encapsulation displacing conventional naked islet treatment for the possible cure of T1DM.

**Conclusion**

Current progress in capsule biocompatibility has brought encapsulation strategies close to wider clinical application, with the hope of transplanting islets close to blood vessels in pre-vascularized solid supports to increase long-term survival. Advantageously encapsulation requires minimal surgery, islets have a reduced outer pore size due to poly-amino-acid coating, capsules can become neovascularized to prevent hypoxia, and the need for toxic immunosuppressive drugs has been eliminated. Nonetheless, the physiological attractiveness of β-cell encapsulation is beset by challenges including paucity of islet transplants, instant blood-mediated inflammatory responses and the inevitable confinement by fibrotic overgrowth. Comparatively, bihormonal closed-loop delivery systems have shown promise in small clinical trials particularly with the use of pramlintide and PK customised algorithms but are behind in terms of development due to a number of limitations. These include the invasive procedure of device transplantation causing inflammation that gives inaccurate CGM data, the requirement for frequent maintenance and changing of system components, the inadequate formulations of insulin and glucagon and the suboptimal accuracy and reliability of CGM with transient deviations including sensor overestimation.

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**Author biography**

In summer 2015 I graduated from Keele University, having achieved a first class honours degree in Biomedical Science. I have always had a personal interest in diabetes, experiencing family members and friends diagnosed with the disease constantly striving to manage their condition. Following my desire to continue to learn about the treatment of different diseases and the safety of pharmaceutical drugs, I am currently studying for an MSc in Toxicology at the University of Birmingham.

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