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Review:

Treatment of diabetes with encapsulated pig islets: an update on current developments*

Hai-tao ZHU[†1,2], Lu LU[2], Xing-yu LIU[3], Liang YU[2], Yi LYU[2,4], Bo WANG[†‡2]

([1]*Heart Center, Northwest Women's and Children's Hospital, Xi'an 710061, China*)

([2]*Department of Hepatobiliary Surgery, the First Affiliated Hospital, Medical College, Xi'an Jiaotong University, Xi'an 710061, China*)

([3]*Department of Hand Surgery, China-Japan Union Hospital, Norman Bethune Health Science Center, Jilin University, Changchun 130033, China*)

([4]*Institute of Advanced Surgical Technology and Engineering, Xi'an Jiaotong University, Xi'an 710061, China*)

[†]E-mail: zht0915@163.com; bobwang75@yeah.net

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Abstract: The potential use of allogeneic islet transplantation in curing type 1 diabetes mellitus has been adequately demonstrated, but its large-scale application is limited by the short supply of donor islets and the need for sustained and heavy immunosuppressive therapy. Encapsulation of pig islets was therefore suggested with a view to providing a possible alternative source of islet grafts and avoiding chronic immunosuppression and associated adverse or toxic effects. Nevertheless, several vital elements should be taken into account before this therapy becomes a clinical reality, including cell sources, encapsulation approaches, and implantation sites. This paper provides a comprehensive review of xenotransplantation of encapsulated pig islets for the treatment of type 1 diabetes mellitus, including current research findings and suggestions for future studies.

Key words: Encapsulation, Pig, Islet, Xenotransplantation, Diabetes mellitus
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1 Introduction

Pancreatic islet transplantation is a viable and attractive option for the treatment of type 1 diabetes mellitus (T1DM) (Shapiro *et al.*, 2000; Leitão *et al.*, 2008; Hatziavramidis *et al.*, 2013; Ramesh *et al.*, 2013). Due to a shortage of human donors, the sourcing of xenogenic islets from pig donors has emerged as an alternative strategy for transplantation. The pig is considered as the suitable islet donor candidate with advantages of the structural similarity between the insulin of pigs and humans, the lack of amyloid formation, resistance to recurrent autoimmunity, and feasibility for genetic immunomodulation (Koulmanda *et al.*, 2003; Yonekawa *et al.*, 2005; Potter *et al.*, 2010; Wynyard *et al.*, 2014; Zhu *et al.*, 2014a; 2014b). Recently, the demonstration of sustained diabetes reversal and prolonged survival of islet grafts (from wild-type or genetically modified pigs) in immunosuppressed diabetic non-human primates (NHPs) (van der Windt *et al.*, 2009; Thompson *et al.*, 2011a; 2011b; 2012; Bottino *et al.*, 2014) has signified a major step in the advance of the use of pig islets as a promising cellular therapy for the treatment of T1DM. However, due to immune incompatibility, xenogeneic rejection is still a major challenge in the application of pig islet xenotransplantation (Scalea *et al.*, 2012). At present, the mainstay of immune-modulatory remedies is the use of heavy and permanent immunosuppressants which have been shown to have harmful effects on both

‡ Corresponding author
* Project supported by the National Natural Science Foundation of China (No. 30700772)
ORCID: Hai-tao ZHU, https://orcid.org/0000-0003-0867-1354

recipients (e.g. opportunistic infection, malnutrition, neuritis, and severe morbidity) and grafts (e.g. islet toxicity). To promote pig islet xenotransplantation into clinical trials safely and effectively, the problems of life-long immunosuppressive agents must first be overcome (Cooper and Casu, 2009; O'Connell *et al*., 2013; Shin *et al.*, 2014). Indeed, only preclinical studies in which the NHP recipients do not need continuous immunosuppressive therapy are considered as an acceptable and possible basis for a clinical trial (Cooper and Casu, 2009). Immunoisolation, hiding the grafts from the recipients' immune system, fundamentally differs from the conventional strategy of continuing immunosuppressive/tolerance-inducing treatments, and represents a potential and appropriate approach for effectively reducing the immunological barriers to the use of pig islets and for promoting graft survival and functionality (Dufrane *et al.*, 2006b; Elliott *et al.*, 2007; Zimmermann *et al.*, 2007; Dufrane and Gianello, 2012; Sakata *et al.*, 2012).

Immunoisolation is usually achieved by coating islet grafts with semi-permeable membranes consisting of polymer materials, creating what are often referred to as an artificial pancreas (AP) or an encapsulated islet (O'Sullivan *et al*., 2011). The semi-permeable, bio-compatible membranes facilitate the exchange of oxygen, nutrients, insulin, and waste, but protect the islet grafts from the host immune response. With the development of more stable and biocompatible encapsulation systems, pig islet xenografts are able to survive and release insulin for a prolonged period of time, thereby controlling glucose metabolism with a reduction or even the omission of immunosuppressive medication (Table 1). Xenotransplantation of encapsulated pig islets offers the prospect of a practical treatment for insulin-dependent diabetes mellitus (IDDM) (Dufrane and Gianello, 2012; Ramesh *et al.*, 2013; Zhu *et al.*, 2014b). However, several important issues need to be addressed before large scale application can be proposed, for example, seeking or generating a reliable and transplantable source of pig islets/insulin-producing cells, developing an encapsulation approach/technology suitable for large-clinical application, and determining suitable implant sites to facilitate islet engraftment and function. The purpose of this article is to provide a comprehensive review of these topics of pig islet encapsulation.

2 Sources of pig islets

High-quality pig islets contribute a lot to the efficacy and functional duration of an AP. Compared with other variables such as gender and body-weight, donor age seems to have a greater impact on islet size, yield, and functionality (Dufrane *et al.*, 2005; Bottino *et al.*, 2007; Kim *et al.*, 2009). Neonatal pig pancreatic cell clusters (NPCCs) provide additional advantages over adult pig islets (APIs), such as their ease of isolation and purification, resistance to ischemia and inflammation during preparation, low cost, and low level of T-cell response (Nagaraju *et al.*, 2015; Zhu *et al.*, 2014a). After implantation, encapsulated immature pig islets, including NPCCs and fetal pig islet-like cell clusters (FPICCs), can proliferate and differentiate into mature β-cell masses and show excellent metabolic control *in vivo* (Omer *et al.*, 2003b; Foster *et al.*, 2007). Unlike APIs, immature pig islet cells are resistant to the toxic effects of pro-inflammatory cytokines, including tumor necrosis factor-α, interleukin-1β, and interferon-γ (Bai *et al.*, 2002), which may diffuse freely across the hydrogel membranes of the capsules due to their low molecular weight. NPCCs offer an alternative and promising source for islet encapsulation in both preclinical and clinical xenotransplantation (Table 1).

In consideration of the potential risk of zoonosis, islet xenografts should be obtained from specific pathogen-free (SPF) and designated pathogen-free (DPF) pig breeds. Chicago Medical School miniature pigs, New Zealand Auckland Island pigs, and transgenic pigs targeting porcine endogenous retrovirus (PERV) (Elliott *et al.*, 2000; Kim *et al.*, 2007; Semaan *et al.*, 2012; Wynyard *et al*., 2014) represent available donor sources for islet immunoisolation. The genetic modification of islet cells is also a practical method for generating new transplantable grafts with special resistances. For example, the immunoisolation of APIs genetically modified to overexpress antiapoptotic *Bcl-2* gene could significantly reduce islet loss after intraportal infusion (Contreras *et al.*, 2004). The successful application of combined encapsulation and genetic modification technologies opens up a new strategy to improve the outcomes from xenoislet transplantation. In the future, genetically engineered pigs (e.g. transgenic pigs expressing human complement regulators, human heme oxygenase-1, or

Table 1 Preclinical and clinical studies of xenotransplantation of encapsulated pig islets

Study	Islet source	Recipient	Device	Islet amount	Implant site	Immuno-suppressive therapy	Outcome
Sun *et al*., 1996	APIs (sow)	Diabetic Cyno (spontaneously, *n*=9)	Alginate-PLL-alginate microcapsule (250–350 μm, 1.0 islet/capsule)	3×10^4–7×10^4 islet/recipient (one, two, or three transplants)	Peritoneal cavity	No	Recipients: 7/9 achieved normoglycemia (120–803 d); RCs (3 months PT): free of cell overgrowth and physically intact
Elliott *et al*., 2005a	NPCCs	Non-diabetic Cyno	Alginate-PLO-alginate microcapsule (500–600 μm, 1.3 islet/capsule)	2000 IEQs/recipient	Peritoneal cavity	No	RCs (8 weeks PT): with insulin and glucagon positive islets, free of cellular debris coating
Elliott *et al*., 2005b	NPCCs	Diabetic Cyno (STZ induction, *n*=8)	Alginate-PLO-alginate microcapsule	10000 capsulated IEQs/kg (two transplants, 3 months apart)	Peritoneal cavity	No	Recipients: a mean 16% reduction in insulin dosage (24 weeks PT), 1/7 weaned off insulin by 36 weeks; RCs: no gross inflammatory reactions
Dufrane *et al*., 2006b	APIs	Non-diabetic Cyno (*n*=7)	High-M alginate microcapsule (698–707 μm, >90% of well-formed shape)	15000 capsulated IEQs/kg	Kidney capsule	No	Recipients: detected levels of pig C-P ((0.14±0.08) ng/ml, 30 d PT); RCs (135 and 180 d PT): with dithizone positive islets and no graft fibrosis
Elliott *et al*., 2007	NPCCs	Patient with T1DM (*n*=1)	Alginate-PLL-alginate microcapsule	15000 capsulated IEQs/kg	Peritoneal cavity	No	Recipient: insulin dose reduced by 30% (12 weeks PT), urinary pig C-P levels remained 0.6 ng/ml for 11 months; RCs (9.5 years PT): with intact shape, contained alive and functional islets
Dufrane *et al*., 2010	APIs	Diabetic Cyno (STZ induction, *n*=5)	Alginate MCD macrocapsule	3–5 MCDs/recipient (about 30000 IEQs/kg)	Subcutaneous tissue	No	Recipients: DM correction for a maximum of 6 months (FBG <107 mg/dl), HAb1c normalization (<7%, 16 weeks PT); RCs: with viable islets, no macroscopic fibrosis or complement deposition
Valdes-Gonzalez *et al*., 2010	NPCC+ Sertoli cells	Adolescent patients with T1DM (*n*=23, two patients withdraw from study)	Collagen-generating macrodevice	4 devices/recipient (250000 IEQs/device, 3 infusions)	Subcutaneous tissue	No	Recipients: insulin dose reduced by 33%, urinary pig C-P levels remained detectable (0.62 μg/24 h), reduced incidence of diabetic complications; RCs: not observed
Vériter *et al*., 2014	APIs+pig MSCs	Diabetic Cyno (STZ induction, *n*=10)	Alginate MCD macrocapsule	15000–625000 IEQs/kg	Subcutaneous tissue	No	Recipients: DM correction for a maximum of 32 weeks (mean FBG <100 mg/dl), better HAb1c correction (7.4%, 28 weeks PT); RCs (30 weeks PT): insulin-positive staining

APIs: adult pig islets; Cyno: cynomolgus monkey; PLL: poly-L-lysine; RCs: retrieved capsules; PT: post transplantation; NPCCs: neonatal pig pancreatic cell clusters; PLO: poly-L-ornithine; IEQ: islet equivalent; STZ: streptozotocin; M: mannuronic acid; C-P: C-peptide; T1DM: type 1 diabetes mellitus; MCDs: monolayer cellular devices; DM: diabetes mellitus; FBG: fasting blood glucose; MSCs: mesenchymal stem cells

Table 2 Devices configuration for encapsulation of pig islets

Device	Pig islet used	Biocompatible encapsulation material	Implantation site	Preclinical studies (in diabetic NHPs)	Clinical trials (in diabetic patients)	Maximum pig-islet survival and diabetes reversal (from preclinical study)	Advantages (for large-scale application)	Limitations
Intravascular device	YPI (6-month-old)	PAN-PVC	Iliac vessels, APF, FCV	ND	ND	ND	Close contact with blood stream; ample supplies of oxygen and nutrient; rapid glucose stimulated insulin secretion	Development of thrombosis; intensive anticoagulation treatment; high risks of vascular prosthetic surgery
Macrocapsule	API, YPI, NPCC	Alginate (suitable), PTFE, acrylic copolymer, agarose, PSU, APCN membrane	Peritoneal cavity, subcutaneous	D	D	Maximum of 32 weeks normalization of glycemia (FBG <100 mg/dl), and mean 28 weeks correction of HbA1c (7.4%) after subcutaneous implantation of alginate based MCD; Up to 30 weeks observation of insulin positive cells after graft removal	Easy implantation and retrieval	Inadequate graft oxygenation; limitation of nutrient/waste transport; low islet seeding density; risk of capsular rupture
Microcapsule	API, YPI, NPCC, FPICC	Alginate with/without PLL/PLO coating (suitable), agarose, NaCS	Peritoneal cavity, subcutaneous, kidney subcapsular	D	D	Insulin independent for a maximum of 803 d with normoglycemia after intraperitoneal injection of alginate/PLL/alginate capsules; Up to 6 months of survival of graft (alginate microencapsulation) in kidney subcapsular of nondiabetic primate	Increased oxygen and nutrient transport; mechanical stability; ease of fabrication; simple injection into peritoneum	Difficulty of removal if necessary; increased graft volume after encapsulation; possibility of capsule clumping (in peritoneal cavity)
Conformal coating	API, NPCC	PEG, alginate, heparin, or multilayer of biomaterials	Liver (intraportal infusion)	ND	ND	ND	Reduction of graft volume and capsule thickness; close contact with blood stream (intraportal); much better diffusion profile and islet oxygenation	No stability for long-term graft immunoprotection; additional systemic immunosuppression

NHPs: non-human primates; YPI: young pig islet; PAN-PVC: polyacrylonitrile-polyvinylchloride copolymer; APF: arteria profunda femoris; FCV: forearm cubital vein; ND: not detected; API: adult pig islet; NPCC: neonatal pig pancreatic cell cluster; PTFE: polytetrafluoroethylene; PSU: polysulfone; APCN: amphiphilic conetwork; D: detected; FBG: fasting blood glucose; HbA1c: glycated hemoglobin; MCD: monolayer cellular device; FPICC: fetal pig islet-like cell cluster; PLL: poly-L-lysine; PLO: poly-L-ornithine; NaCS: sodium cellulose sulfate; PEG: polyethylene glycol

knocking-out tissue factors, or multi-transgenic pigs) will emerge as promising donor sources for islet immunoisolation in preclinical and clinical applications with advantages of low antigenicity, resistance to inflammation or complement mediated islet damage or loss, and sustained islet survival and functionality.

3 Encapsulation approaches

The introduction of the concept of immunoisolation dates back to 1933. Since then, several different types of immunoisolation devices have been created and studied (Table 2). Overall, the designs of encapsulation systems can be divided into two major categories: intravascular and extravascular devices.

3.1 Intravascular devices

In intravascular devices, islet grafts are enclosed in a large semi-permeable chamber containing a number of small diameter artificial capillaries made of polyacrylonitrile-polyvinylchloride copolymer (PAN-PVC) (Lanza *et al.*, 1996; Borg and Bonifacio, 2011). For implantation, this device is connected directly to the circulatory system of the recipient by vascular anastomosis. The close contact between the islets and the host's blood stream ensures a rapid exchange of insulin and glucose, thereby inducing a strict and prompt regulation of blood glucose (BG). In diabetic dogs, intravascular devices containing pig islets (160000–430000 islet equivalents (IEQs)/device) provided good glycemic control for more than eight months without immunosuppression (Maki *et al.*, 1996). However, thrombus formation in the lumen of the intravascular device or at the anastomotic site proved to be a major obstacle, in spite of anticoagulant therapy in massive doses. Also, it is quite plausible that the relatively high flow-rates through the device could not allow an adequate exchange of nutrients to sustain the prolonged survival and favorable function of islet grafts. Complications (e.g. bleeding, intimal hyperplasia, and infection) associated with vascular prosthetic surgery still remain a serious threat limiting the therapeutic potential of this approach. Thus, research on intravascular devices has remained on hold since the 1990s (Petruzzo *et al.*, 1991; Maki *et al.*, 1996; Maki and Monaco, 1997). But in 2008, Prochorov *et al*. (2008) conducted an intravascular implantation of fetal rabbit islets (>6000 IEQs/kg) contained in nylon microporous macrocapsules into the arterial-venous fistulas of T1DM patients without immunosuppression. All recipients were given the current standard antithrombotic therapy. After two years of follow-up, the authors reported a significant reduction in exogenous insulin demands, together with increases in C-peptide and immunoreactive insulin levels in 14 of the 19 patients. Moreover, neither vascular lumen narrowing nor thrombosis was observed. This encouraging achievement raises the prospect that new biomaterials with new intravascular approaches may lead to good outcomes, which will produce a clinically relevant intravascular device.

3.2 Extravascular devices

This type of device does not require anastomosis when it is transplanted into the recipient and therefore has an advantage over intravascular devices in terms of preclinical and clinical applications (Table 2). Usually, extravascular devices are categorized into two main types by their sizes: macroencapsules (as large as 3 cm×8 cm) and microencapsules (ranging from 150 to 1000 μm) (de Vos *et al.*, 2010; Buder *et al.*, 2013).

3.2.1 Macrocapsules

Macrocapsules contain large numbers of islet grafts within a tubular diffusion chamber or planar chamber. One advantage of macrocapsules is that they can be implanted and removed with minimal risk. On the other hand, their major drawback is the limited oxygen diffusion and nutrient transport, which tends to result in impaired viability, dysfunction, or even central necrosis in islets (Beck *et al.*, 2007; Weir, 2013). Current research on macroencapsulation systems focuses largely on the development of techniques and configurations, which can promote neovascularization and provide sufficient oxygen and nutrition for islet cells (Grundfest-Broniatowski *et al.*, 2009; Dufrane *et al.*, 2010; Barkai *et al.*, 2013; Vériter *et al.*, 2014; Scharp and Marchetti, 2014).

3.2.1.1 Current approaches

A commonly used commercial macrocapsule is the TheraCyte device, which is made of bilayered polytetrafluoroethylene (PTFE) membranes (Sörenby

et al., 2008; Malavasi *et al.*, 2010; Kirk *et al.*, 2014). The outer membrane of the TheraCyte device is designed for strength and to facilitate neovascularization, and the inner membrane provides immune protection. In diabetic mice, subcutaneous transplantation of NPCCs encapsulated in the TheraCyte device greatly reversed diabetes for up to 10 weeks. In non-diabetic monkeys, histology of the retrieved device showed that it had no coating of cellular debris and no inflammatory reaction was observed in the adjacent tissues (Elliott *et al.*, 2005a). The TheraCyte device is impermeable to immune cells, but the pore size suggests that the membrane may be permeable to antibodies and complements. The absence of pig islet damage inside the device can possibly be explained by the slow passage of IgG antibodies and little expression of α galactose antigens (activators of acute rejection) on islet cells (Kin *et al.*, 2000; Marigliano *et al.*, 2011; Kumagai-Braesch *et al.*, 2013). Furthermore, a novel implantable macrochamber (β-Air device) has been created to offer immune protection and an adequate oxygen supply for islet grafts (Ludwig *et al.*, 2010; Barkai *et al.*, 2013). This disc-shaped combinational device consists of two compartments, an oxygen supply compartment and an immune protected compartment containing islet grafts immobilized in alginate hydrogel. Ludwig *et al*. (2010) demonstrated that API allografts remained morphologically intact, viable, and functional for significant times within the double-chambered bioreactor connected to subcutaneous refueling ports through which an oxygen-CO_2 mixture was delivered by daily injection. With structural improvements (e.g. increased islet mass (up to (4160±380) IEQs/cm^2), a better gas ventilation system, and an improved immune barrier) and successful applications in large animals (Ludwig *et al.*, 2013; Neufeld *et al.*, 2013), the β-Air device provides a potential alternative strategy for preclinical pig islet xenotransplantation. Nevertheless, the primary obstacle is that the cell density in this device needs to be quite low to ensure an adequate oxygen supply. This indicates that if large numbers of pig islets (25000–100000 IEQs/kg) are required to achieve insulin independence in diabetic NHPs (Hering *et al.*, 2006; Casu *et al.*, 2008; van der Windt *et al.*, 2009; Thompson *et al.*, 2011a; 2012; Zhu *et al.*, 2014a), numerous or larger devices must be implanted. However, it is impossible to find a suitable surgical site to accommodate such macrodevices. The challenge will be settled if approaches can be found to offer more oxygen by improved delivery methods or better vascularisation.

More recently, a monolayer configuration (made of alginate) of macroencapsulated APIs implanted subcutaneously showed the ability to correct hyperglycemia for up to six months in diabetic monkeys without immunosuppression (Dufrane *et al.*, 2010). In this device, pig islets were seeded as a monolayer on a human decellularized collagen matrix (mean 50000 IEQs/cm^2) to improve the number of islets per unit surface area and to enhance biological support (e.g. oxygen delivery and nutrient exchange). Subsequently, a better and longer diabetic control (up to 32 weeks) was obtained in diabetic monkeys after subcutaneous implantation of APIs and mesenchymal stem cells (MSCs) which were co-encapsulated in the same monolayer device (Vériter *et al.*, 2014). The co-transplantation of MSCs significantly improved the vascularization (neoangiogenesis) and oxygenation of the macrodevice in terms of an increased number of vessels and elevated generation of vascular endothelial growth factor (VEGF). Also, the pig MSCs could be expanded and differentiated, therefore potentially constituting an alternative, renewable, and continuous source of insulin-producing cells. A phase I clinical trial is ongoing in Belgium to further investigate the safety and effectiveness of this monolayer cellular device for allotransplantation of encapsulated islets into humans.

3.2.1.2 Clinical trials

Valdés-González *et al.* (2005) reported acceptable clinical outcomes from xenotransplantation of NPCCs encapsulated in an autologous collagen-generating device with homologous Sertoli cells, which had excellent immunomodulatory properties. Prior to islet-Sertoli cell infusion (30–100 Sertoli cells per islet), the macrodevices (6 cm×0.8 cm) consisting of two steel mesh tubes and a PTFE rod interior were implanted subcutaneously for two months to allow tissue ingrowth and vascularization. Without the use of any immunosuppressants, 6 of 12 patients showed a 50% or greater reduction in exogenous insulin requirements, and this reduction was maintained for up to four years. Three years post-implantation, histological samples of tissue extracted

from the device stained positive for insulin-producing cells. Moreover, routine microbiological screening of the recipients was constantly negative. With technical improvements, Valdés-González *et al.* (2007) further demonstrated the safety and feasibility of this xenotransplantation procedure for the control of T1DM in a single detailed case study. Valdes-Gonzalez *et al.* (2010) reported a longitudinal study of 23 T1DM patients xenotransplanted with collagen macroencapsulated NPCC-Sertoli cells between 2000 and 2004. All recipients produced detectable porcine C-peptide in their urine and more than 50% of recipients presented a greater reduction (>33%) in their exogenous insulin requirement. Unfortunately, this pilot study did not contain a diabetic control undergoing tightly controlled treatment to validate the results.

3.2.1.3 Some considerations

Although interesting data have come from macroencapsulation studies, additional issues have to be addressed before the macroencapsulation of pig islets can emerge as a practical clinical option for the treatment of T1DM. (1) Macrocapsules with optimal geometry and structure are needed to enhance the supply of oxygen and nutrients for the contained pig islets, and to minimize the diffusion distance for insulin and glucose. (2) Studies have demonstrated that macroencapsulation of pig islets can result in normoglycemia in diabetic recipients; however, very little is known about the kinetics of insulin release from the encapsulated pig islets. Is there an adequate and timely insulin response to changes in glucose levels? (3) Vascularization of the membrane, a mandatory process for favorable function and prolonged survival of macroencapsulated islets, is preceded by inflammation which involves the recruitment of inflammatory cells around the macrodevices and the formation of an extracellular matrix to facilitate ingrowth of endotheliocytes (de Vos *et al.*, 2010). It is essential to explore which factors need protection in a macrodevice during the early and late implant stages. (4) What is the optimal amount of pig islet grafts that can be well supported by a certain surface (i.e. the optimal packing density of islet cells) that receives oxygen and nutrient supply from the peripheral tissues?

3.2.2 Microcapsules

Microcapsules incorporate individual or small groups of islets in a spherical hydrogel polymer with a stable mechanical structure. A number of considerations favor microcapsules over macrocapsules (Table 2). The spherical geometry and low volume of microcapsules offer better oxygen and nutrient transport due to a higher surface area-to-volume ratio (van Schilfgaarde and de Vos, 1999; Beck *et al.*, 2007). Furthermore, microcapsules require less complex or expensive manufacturing procedures, and can be simply injected without major surgery (Scharp and Marchetti, 2014). Their primary drawback is that it is difficult to remove them completely, especially if there is pericapsular fibrotic overgrowth (PFO) after implantation. Nevertheless, a novel method, attachment of microcapsules to a plasma-treated polydimethylsiloxane (PDMS) sheet, appears to be applicable for retrieving microencapsulated pig islets when required (Shin *et al.*, 2013).

3.2.2.1 Current approaches

Currently, there seems to be a great deal more researches and developments on microdevices than on macrodevices in preclinical pig islets xenotransplantation (Table 1). In this area, alginate is the most suitable and commonly used biomaterial to entrap islet cells (de Vos *et al.*, 2014; Orlando *et al.*, 2014) (Tables 1 and 2). In general, islet-containing alginate solution is dropped through a nozzle into calcium or barium solution to generate a microbead incorporating an individual or few islet grafts. The surface of the device can be further coated with polycation/alginate to confer better permselectivity and mechanical stability. Moreover, a variety of capsule sizes and structures are available, including a core-shell version, double core/shell version, and a solid-like/liquid core version. However, a lack of standardized formulations contributes greatly to current reported lab-to-lab variation in biocompatibility and immune-protection of microencapsulation systems.

Several studies have demonstrated the successful protection of alginate-microencapsulated pig islets against immune destruction and long-term reversal or control of hyperglycemia (ranging from four months to over 450 d) in diabetic rodents (Lanza *et al.*, 1999; Omer *et al.*, 2003b; Foster *et al.*, 2007; Cui *et al.*,

2009). More importantly, studies describing xenotransplantation of pig islets (APIs or NPCCs) microencapsulated in an alginate-matrix confirmed their biocompatibility and safety (no evidence of porcine viral transmission) in nondiabetic NHPs (Elliott *et al.*, 2005a; Dufrane *et al.*, 2006b), and demonstrated their capacity to achieve metabolic control and reduce insulin requirements in diabetic dogs and NHPs (Sun *et al.*, 1996; Elliott *et al.*, 2005b; Abalovich *et al.*, 2009). However, the clinical application of microencapsulated pig islets is not yet supported by more solid preclinical achievements. Only one historical study (Sun *et al.*, 1996) indicated that spontaneously diabetic monkeys could become insulin independent for periods ranging from 120 to 803 d with normalized fasting BG levels following 1–3 transplants of microencapsulated APIs. Although the data obtained were encouraging for clinical practice, they might be largely dependent on the primate model. To achieve long-term biocompatibility and viability (e.g. >6 months) and favorable immunoprotection of alginate microencapsulated pig islets in primate recipients, several suggestions should be considered (Sun *et al.*, 1996; Dufrane *et al.*, 2006b; Calafiore and Basta, 2014): (1) the use of donor pigs with a well-defined genetic background; (2) the use of pig islets with high-purity (>90% purity); (3) fabrication of microcapsules using highly purified material with improved stability, low heavy metal, protein and endotoxin content, and a "clinical-grade" basic alginate is recommended; (4) the culture of microencapsulated islets in a medium containing 1.8 mmol/L $CaCl_2$ for 18 or 24 h prior to implantation; and (5) the transplantation of grafts composed of more than 90% well-shaped capsules (of regular and spherical shape).

3.2.2.2 Clinical trials

Elliott *et al*. (2007) first reported a case of long-term survival (>9.5 years) of microencapsulated NPCCs in a male patient with T1DM who received a single implantation of alginate based grafts (15000 IEQs/kg) into the peritoneal cavity in 1996. Following transplantation, the daily insulin dose was reduced by up to 30% and the urinary porcine C-peptide remained detectable for over one year. Ten years later, laparoscopic examination revealed living and functional NPCCs in his abdomen, and no evidence of xenosis or gross peritoneal reaction or fibrosis was observed. In 2007, a larger clinical study of commercial microencapsulated pig islets (also called "Diabecell") was conducted by the Living Cell Technologies (LCT) Company (Tan, 2010) (DIABECELL® is currently in late-stage clinical trials. Further information is available from http://www.lctglobal.com/products/diabecell/development-to-date). After implantation (5000–10000 IEQs/kg), two patients were completely independent of insulin administration for up to 32 weeks, and six patients displayed improved BG control as reflected by their reduced glycated haemoglobin (HbA1c) levels and daily insulin dose. Currently, other phase IIa trials are being conducted in New Zealand and Argentina.

3.2.2.3 Some considerations

All these clinical trials cannot be considered as true breakthroughs for the treatment of T1DM, since no exogenous insulin administration was interrupted and the metabolic control was not excellent. Nevertheless, these pilot clinical trials confirmed the safety and potential therapeutic effects of microencapsulated islet xenografts in human recipients. Improvements in microcapsule design and fabrication, coupled with the emergence of sufficient or renewable islet cell mass with low xenoantigenicity and high-quality (e.g. high purity, with inflammation- and hypoxia-resistance), as well as advances in optimized biomaterials and the bioengineering of implantation sites may help to provide a favorable and stable metabolic control in diabetic patients.

3.3 Future directions: conformal coating

Traditional methods of islet microencapsulation can still result in diffusional limitations associated with capsules of larger size (>600 μm in diameter), which may lead to blunted insulin secretion in response to glucose, and even core hypoxia or necrosis of islets. In addition, the mean diameter of islets is about 150 μm, increasing the total volume of the implant by dozens of times after microencapsulation. Usually, transplant sites able to accommodate such large volumes of implants are confined to the peritoneal cavity (Sun *et al.*, 1996; Elliott *et al.*, 2005b; 2007; Cui *et al.*, 2009), which is poorly vascularized and constitutes a preferential location for inflammatory and immunological reactions (Hsu *et al.*, 1999; Omer

et al., 2003a; de Groot *et al.*, 2004). Thus, intraperitoneal injection seems less appropriate for promoting engraftment of encapsulated pig islets (Dufrane *et al.*, 2006a).

To address these issues, investigators developed an approach to encapsulate islets by conformal coating, which may increase capsule stability, minimize capsule thickness and size and graft volume, and allow graft transplantation into the liver through the portal vein (Teramura and Iwata, 2008; 2009; Kizilel *et al.*, 2010; Tomei *et al.*, 2014). Conformal coating can be defined as the application of hydrogels to the surface of an islet cell by interfacial polymerization to form a cross-linked hydrogel and a thin (<50 μm) coating (Cruise *et al.*, 1998; Sefton *et al.*, 2000; Wilson *et al.*, 2008; Scharp and Marchetti, 2014). Islet surface modification with the biologically inert, amphiphilic polymer polyethylene glycol (PEG) has emerged as a promising and alternative approach. Not only did PEGylation have no adverse effects on islet morphology, viability, or functionality (Cruise *et al.*, 1998; Contreras *et al.*, 2004; Teramura and Iwata, 2009), but it was also found to prevent islet recognition by activated immune cells *in vitro* (Lee *et al.*, 2004) and to reduce islet allograft damage or loss after intraportal transplantation (Teramura and Iwata, 2009). However, some *in vivo* studies suggested that islet surface modification alone, either with a PEG or heparin coating, was not a very stable immunoprotective method since combinatory treatments of low-dose immunosuppressants (e.g. cyclosporine or anti-co-stimulatory antibodies) had highly synergic effects on the maintenance of normoglycemia and inhibition of sensitized host immune responses (Lee *et al.*, 2006a; 2006b; Jung *et al.*, 2012; Jeong *et al.*, 2013). It is unlikely that this technology will prove to be highly effective and applicable in pig islet xenotransplantation using present methods. Intraportal infusion of APIs (5000 IEQs/recipient) modified with PEG derivatives into non-obese diabetic/severe combined immune-deficient (NOD-SCID) mice gave better glucose control, but the euglycemia (non-fasting glucose <200 mg/dl) was very transient (<2 weeks) (Contreras *et al.*, 2004). In a study by Cabric *et al.* (2007), although transplantation of APIs (7500 IEQs/kg) coated with heparin into the livers of piglets resulted in lower insulin release (an indicator of cell damage), as well as decreased thrombin antithrombin (TAT) and C3a generation, the observation period was too short (<60 min) and long-term graft viability/functionality was not assessed.

Currently, by using the layer-by-layer (LbL) method, it is possible to fabricate complex coatings (e.g. PLL-g-PEG-biotin/streptavidin, chitosan/alginate, PEG-*N*-hydroxysuccinimide/alginate, or PEG-complement receptor 1/heparin multilayer films) of nanometer thickness to significantly improve the stability of the layers, enhance nutrient diffusion, promote growth of new microvasculature, inhibit complement activation/blood-mediated inflammatory responses, and prolong islet graft survival (Cabric *et al.*, 2007; Teramura and Iwata, 2008; Wilson *et al.*, 2008; 2011; Zhi *et al.*, 2012; Gattás-Asfura and Stabler, 2013; Luan and Iwata, 2013; Scharp and Marchetti, 2014). The main drawback of this innovative approach is the possible cytotoxicity of the compounds used. Thus, it is necessary to develop new methods to fabricate novel multifunctional coatings with excellent immunomodulatory capacities which can facilitate the reconstruction of the microenvironment (e.g. provide extracellular matrix support) and satisfy the physical demands of islet grafts. In this sense, the LbL strategy is still able to offer an opportunity to combine the inherent advantages of microencapsulation and conformal coating. In the future, the use of LbL with multifunctional materials derived from non-toxic biologically-active polymers or living regulatory cells will serve as a useful approach for sustained and favorable islet viability and functionality.

4 Implantation sites

The microenvironment of the implant site plays a major role in engraftment and survival of encapsulated pig islets xenografts. An optimal site should provide (1) a simple and safe implanting/removal operation, (2) immune protection, (3) a physiological route for insulin delivery, (4) a sufficient blood and oxygen supply, (5) enough space for a large volume of encapsulated islets, and (6) compatibility with immunoisolation systems (Zhu *et al.*, 2014b).

Due to its lower restriction on the volume of grafts, intraperitoneal space has been used most often for the transplantation of encapsulated islets (Vériter *et al.*, 2013), especially in xenotransplantations of

encapsulated pig islets into experimental or preclinical models (Lanza *et al.*, 1991; Sun *et al.*, 1996; Kin *et al.*, 2002; Omer *et al.*, 2003a; Elliott *et al.*, 2005a; 2005b; 2007; Vinerean *et al.*, 2008; Grundfest-Broniatowski *et al.*, 2009). Although the procedure is easy and less invasive by laparoscopy, the peritoneal cavity may not be the ideal location for islet engraftment. Drawbacks include the lack of close contact with the bloodstream and the difficulty of retrieving the capsules if needed. Moreover, transplantation of encapsulated islets into the peritoneum may aggravate hypoxia and hamper the insulin secretory response since oxygen and insulin delivery through the peritoneal cavity is by passive transport only (de Groot *et al.*, 2004). Studies in rodent models have demonstrated that macrophages and lymphocytes in the peritoneum are directly involved in the cellular infiltration, PFO, and rapid degradation of the capsule (Omer *et al.*, 2003a; Dufrane *et al.*, 2006a; Vaithilingam *et al.*, 2013). Despite the administration of immunosuppressive agents showing beneficial effects in improving the biocompatibility and prolonging the survival of encapsulated pig islets (either via macroencapsulation or microencapsulation) after intraperitoneal implantation (Omer *et al.*, 2003a; Safley *et al.*, 2005), the combination of immune suppression and encapsulation potentially reduces to nihil the interest in encapsulation (Orlando *et al.*, 2014).

By contrast, subcapsular kidney (suitable only for microcapsules) and subcutaneous spaces (suitable for different encapsulation devices) showed weaker cellular reactions, better islet viability, and fewer broken capsules than the peritoneal cavity for transplantation of encapsulated pig islets (Dufrane *et al.*, 2006a), rendering them interesting alternative sites for receiving encapsulated pig islets in preclinical studies (Elliott *et al.*, 2005a; Dufrane *et al.*, 2006b; 2010). Pre-vascularization of the implant site or co-encapsulation of pig islets and MSCs, especially for a subcutaneous space, is a very useful strategy to promote neovascularization around the implanted devices and to reduce hypoxic stress in the capsulated islets (Wang *et al.*, 2002; 2003; Vériter *et al.*, 2014). Nowadays, with the emergence of conformal coating and surface modification technology, the liver is again being investigated as a possible implant site for both allo- and xeno-islet grafts due to the resultant reduction in graft volume, prevention of instant blood-mediated inflammatory reaction (IBMIR) and intraportal thrombosis, and improvement in graft survival (Cabric *et al.*, 2007; Teramura and Iwata, 2008; 2009; Luan and Iwata, 2013).

Other novel sites reported recently, such as striated muscle (Christoffersson *et al.*, 2010; Espes *et al.*, 2011) and bone marrow (Meier *et al.*, 2014), represent feasible locations for the transplantation of microencapsulated or conformal coated islets with the advantages of good vascularization and relatively easy access. Nevertheless, further studies need to be performed in large animal models to evaluate the long-term graft survival and function in such sites.

5 Conclusions

Xenotransplantation of encapsulated pig islets may overcome the two major hurdles of conventional islet transplantation: limited human donor supply and the extensive use of immunesuppressants. Though studies conducted in both animal models and human recipients (early phase clinical trials) have demonstrated the feasibility of encapsulated pig islet xenontransplantation in the treatment of T1DM, clinical application is still a long way off. The successes are difficult to replicate (lab-to-lab variation) and there is a lack of a standard protocol for the preparation, engineering, and implantation of encapsulated islet xenografts. A consensus among specialists needs to be reached to further advance the current encapsulation technology. Among the encapsulation approaches, in our opinion, conformal coating of micron and submicron scale on individual islets or cell aggregates represents a promising direction. In the near future, with advances in genetically modified pig islets or stem-cell derived functional islets, improvements in encapsulation design and process, the emergence of novel biocompatible encapsulation materials and well bioengineered microenvironments for graft colonization, and greater preclinical and clinical experience with xenotransplantation will probably provide a clinically useful means of achieving β-cell replacement for IDDM.

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Compliance with ethics guidelines

Hai-tao ZHU, Lu LU, Xing-yu LIU, Liang YU, Yi LYU, and Bo WANG declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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中文概要

题　目：胶囊猪胰岛移植治疗糖尿病：现况与进展

概　要：本文对胶囊猪胰岛异种移植治疗糖尿病的相关研究进行综述，展示目前的研究现状，指出目前存在的问题，并提出未来的研究方向。基于包被胰岛的体积、活性、功能及生物安全性等重要决定因素，对猪胰岛来源、胶囊包被技术/方法及植入部位的选择进行了对比性、系统性地阐述，具有较高的参考价值。胶囊猪胰岛异种移植治疗糖尿病具有良好的应用前景，小胶囊或保形包被技术或是今后的发展方向。

关键词：胶囊包被；猪；胰岛；异种移植；糖尿病