Advanced Drug Delivery Reviews 67-68 (2014) 84–92

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Clinical application of microencapsulated islets: Actual prospectives on progress and challenges ☆

Riccardo Calafiore *, Giuseppe Basta

Department of Internal Medicine, University of Perugia, Via Enrico dal Pozzo, s.n.c., Perugia 06126, Italy

ARTICLE INFO

Available online 1 November 2013

Keywords:
Diabetes
Alginate
Microcapsules
Therapy

ABSTRACT

After 25 years of intense pre-clinical work on microencapsulated intraperitoneal islet grafts into non-immunosuppressed diabetic recipients, the application of this procedure to patients with type 1 diabetes mellitus has been a significant step forward. This result, achieved in a few centers worldwide, underlies the safety of biopolymers used for microencapsulation. Without this advance, no permission for human application of microcapsules would have ever been obtained after years of purification technologies applied to the raw alginates. To improve safety of the encapsulated islet graft system, renewed efforts on the capsules' bioengineering, as well as on insulin-producing cells within the capsular membranes, are in progress. It is hoped that advances in these two critical aspects of the cell encapsulation technology will result in wider human application of this system.

Contents

1. Introduction . . . 85
   1.1. Clinical islet cell transplantation . . . 85
2. Immunoprotection of the transplanted islets within selective permeable, nontoxic microcapsules . . . 85
   2.1. Introductory remarks . . . 85
   2.2. General principles . . . 86
   2.3. Alginic polymer qualification for encapsulation for human use (University of Perugia technology) . . . 86
      2.3.1. Protein content . . . 86
      2.3.2. Endotoxin content . . . 86
      2.3.3. Heavy metal content . . . 86
   2.4. Fabrication process . . . 86
   2.5. Pre-clinical trials . . . 87
   2.6. Clinical application of microencapsulated islets for patients with T1D . . . 88
      2.6.1. The University of Perugia experience . . . 88
      2.6.2. The University of New South Wales, Australia experience . . . 88
      2.6.3. The Living Cell Technologies (LCT) experience . . . 89
3. Encapsulated islet allografts in humans with no systemic immunosuppression: advances and critique . . . 89
4. Future directions . . . 90
   4.1. Generation of smaller microcapsules . . . 90
   4.2. Two aqueous-phase emulsification . . . 90
      4.2.1. Alginates . . . 90
      4.2.2. PEG . . . 90
      4.2.3. Interfacial polymerization . . . 90
      4.2.4. Layer-by-layer assembly of polyelectrolyte multilayer . . . 90
5. Alternative sites of implant . . . 91
6. Conclusions . . . 91
Acknowledgments . . . 91
References . . . 91

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Cell encapsulation and drug delivery".

* Corresponding author.
E-mail addresses: riccardo.calafiore@unipg.it, isolette@hotmail.it, r.calafiore@yahoo.it (R. Calafiore).

0169-409X/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.addr.2013.09.020

1. Introduction

Ethiopathogenesis of type 1 diabetes mellitus (T1D) is based on autoimmune β-cell selective killing, driven by autoreactive CD4 clones, also involving a complex cascade of pro-inflammatory and pro-apoptotic molecules. Different working hypotheses on a possible direct role of infection agents on pancreatic (β-cells included) destruction remain unproven [1]. Nevertheless, and regardless of the acquisition of novel β-cell destruction pathways, the near total disappearance of β-cells within the islets leads to endogenous insulin deprivation, which clinically translates into overt diabetes mellitus. Should exogenous insulin supplementation be obviated or delayed, acute and frequently fatal complications would ensue. Hence and so far, exogenous insulin continues to be the mainstay of T1D therapy — virtually the only treatment that allows for T1D patients' survival. In fact, if appropriately modulated, exogenous insulin therapy regimens, implemented by the introduction of the new analogic molecules [2], according to established injection algorithms, may substantially reduce the risk of developing secondary, chronic complications of T1D, with special regard to micro- and macro-angiopathy, a combination that leads invariably to retinopathy, disabling neuropathy, cardiovascular disease and terminal renal failure [2]. However, while life-saving and able to at least restrain the development ofT1D-linked secondary multiorgan damage, insulin therapy is associated with several pitfalls, based on the fundamental principle that exogenous insulin administration can never mimic the stimulus-coupled insulin secretory kinetics of normal β-cells under physiological conditions. Moreover, in a minor cohort of T1D patients, blood glucose (BG) control, despite applied intensive insulin therapy regimens, is brittle, regardless of the insulin delivery system (i.e. conventional vs. minipump). For these reasons and because in general, as mentioned above, BG control using exogenous insulin will be imperfect even in the best conditions, there have been strenuous efforts to substitute the diseased or dead islet β-cells, associated to T1D, with fresh and viable tissue derived from cadaveric pancreatic donors.

While this approach has been pursued for over three decades, using whole donor pancreatic, or isolated islets from donor pancreases, the initial hopes that such a replacement therapy would rapidly and fully succeed have been largely shattered. In fact and in particular, whole pancreatic transplantation remains a quite major surgery, still associated with high morbidity. Isolated islet grafting procedures, *via* transhepatic puncture delivery, in T1D patients, have encountered a series of technical and methodological problems, many of which are still pending. For both procedures, the recipient's systemic pharmacological immunosuppression is a necessary condition for allowing the tissue engraftment and immunologic acceptance. This is not a minor point, considering that β-cell replacement strategies are not a life-saving procedure and per se would not justify the use of dangerous immunosuppressive regimen protocols. Today islet and pancreas transplantation may be reimbursed in some countries, and is even cited in the Clinical Practice Recommendations of the American Diabetes Association [2], intended for a limited cohort of T1D patients; however, both procedures continue to be virtually experimental.

1.1. Clinical islet cell transplantation

Outcomes of clinical islet transplantation (TX) have steadily improved through eras [3] with special regard to 2007–2010, when, according to the data of the Clinical Islet Transplant (CIT) Consortium, 65% of the grafted patients reached insulin-independency at 1 year of post-TX. Less consistent was the maintenance of insulin independence at 5 years of TX — which has significantly improved, reaching 40–50% of all treated patients, only in a few centers worldwide (Edmonton, Minneapolis, Geneva, Lille and Milano) although still unable to match the clinical outcome of whole pancreatic grafts. A major problem is that clinical results obtained by a single whole pancreatic graft may be paralleled by islets obtained from at least 2–3 if not more cadaveric pancreatic donors. In selected instances, review of the CIT Registry (CITR) (www.citregistry.org) data shows that the use of new specific Tc-depleting agents in association with anti-inflammatory molecules allowed extension of insulin independence, in a few cases, by 60–70%. In these successful cases, the pro's supporting the islet TX procedure can be summarized as shown in Table 1.

Such beneficial effects have been deemed to occur, not necessarily in fully insulin-independent patients but also in patients where the TX was functioning, in terms of serum C-peptide detection, and with improvements in HbA1c levels [13,14].

However, in these selected centers, according to CITR, crude mortality associated with the procedure was 3% out of 6 years of elapsed follow-up per patient (including stroke, heart attack, respiratory distress syndrome, ketoacidosis and multiorgan failure); while neoplastic events (0.02/individual/year) also appeared, particularly for lung cancer and skin tumors.

By the same token, several problems, some apparently quite serious, still hamper widespread diffusion of the procedure in a large segment of the T1D population (Table 2).

In summary, these drawbacks remain largely unanswered and restrict islet transplantation to a selected experimental procedure, applicable to only a minor cohort of patients with T1D and available in only a few centers worldwide. To make this approach a cure for T1D, as initially hoped, the gap between dream and reality is yet to be filled.

2. Immunoprotection of the transplanted islets within selective permeable, nontoxic microcapsules

2.1. Introductory remarks

Based on the principle that a possible β-cell substitution cell therapy, envisioned as a cure for T1D, should target all and not only a minority of the patients affected by this metabolic disease, some possible solutions are required. Should grafted islets be enveloped within artificial membranes that selectively regulate cross-permeability of noxious soluble factors, while preventing access to immunoactive cells and molecules, immunosuppressive treatment of the recipients could theoretically be obviated. The physical shield surrounding the islets would attenuate the impact of acute and chronic immune rejection, also offering the opportunity to employ less toxic and possibly locally delivered agents that would generally make the procedure more acceptable.

Additionally, microencapsulation might allow the use of nonhuman tissue as a resource for donor islets, thereby contrasting with the chronic restricted availability of human donor pancreases.

The immunoprotection approach by physical barriers may be pursued by the use of macrodevices or microcapsules, both based on the use of highly selective and nontoxic membranes, variably configured, and comprised of highly purified constituent biopolymers. In the beginning of our research activity on islet immunoprotection, 25 years ago, we selected microcapsules basically made of alginic acid derivatives complexed with amino-acid polycations.

Table 1
Clinical islet transplantation: beneficial effects of quality of life (QOL) and chronic complications.

QOL	Improved	Reference
Cardiovascular	Stabilized/improved	[4] MD Bellin, 2011; [5] T Tharavanij, 2008; [6] P Fiorina, 2005.
Renal	Prevention of GFR decline	[7] Thompson DM 2011; [8] Leitao CB 2009.
Neuropathic	Stabilized/improved	[9] Del Carro U 2007; [10] Lee TC 2005.
Retinal	Stabilized/improved	[11] Warnock GL 2008; [12] Thompson DM 2008.

Table 2
Clinical islet transplantation: adverse factors hampering success of intra-hepatic islet allograft procedures.

a. Too many islets (IEQ)/recipient required to achieve substantial functional TX-related clinical effects.
b. Too many islets (IEQ)/recipient required to achieve substantial functional TX-related clinical effects.
c. Sub-optimal, even if improved, long-term TX functional life-span.
d. Significant toxicity, although improved, of the employed general immunosuppressive protocols.
e. Islet engraftment problems, with special regard to site of TX effects: liver, due to immunological, anatomical [15,16] and physiological [17] factors.
f. Cost effectiveness: still an extremely expensive procedure in relation to the achieved effects.

2.2. General principles

Microencapsulation consists of entrapping cells/tissue within polymeric and non-cytotoxic membranes that constitute immunoprotective barriers. Based on this concept, a wide spectrum of cells, other than pancreatic islets, has been immobilized within the microcapsules which may expand the applicability of this strategy beyond diabetes. However, it was recently postulated that success of this approach may require detailed focus on multiple issues concerning biocompatibility and bioperformance of the microcapsules. Furthermore, microcapsular three-dimensional architecture, based on highly purified and almost endotoxin- and protein-free alginates, has been shown to promote better growth, differentiation and maturation of different cell types, including mesenchymal stem cells, mESCs, hESCs, neural stem cells and hepatocytes [18–21]. The vast majority of alginate-based microcapsules historically measured 600–800 μm in diameter, although generation of small-size alginate capsules is underway.

Table 3
Physical chemical properties of alginate solution.

1.8% sodium alginate solution	
Product description	
Concentration: 1.8% Components • Keltone LVCR Lot.67432A, stored at 2–8 °C light protected • Sodium chloride: 0.9%. Volume: 500 mL Container PET bottle store Storage conditions: Stored at 2–8 °C light protected	
1.8% sodium alginate solution	
Identity and composition	
Parameters	Results
Molecular weight	120,000–190,000 kDa
Mannuronic/guluronic acid ratio	Mannuronic acid (M) content: M fraction (F_M) 61% Guluronic acid (G) content: G fraction (F_G) 39%.
Viscosity: 1.8%	100–300 cps
pH	T 20 °C = 7.2–7.6
Microbiology (bacteria, yeast, fungi)	Sterile
Endotoxin (EU/mL)	<0.5 EU/mL
Protein content	2–2.5 μg/mL
Chemical analysis	Ca: <100 ppm Cu: <40 ppm Fe: <60 ppm Hg: <40 ppb Mg: <40 ppm Zn: <40 ppm Pb: <50 ppm Si: <10 ppm Mn: <10 ppm Sr: <40 ppm As: <100 ppb

Other polymers, such as polyethylene glycol (PEG) [22,23], agarose [24] and HEMA-MMA (hydroxyethyl methacrylate-co-methyl methacrylate) [25,26], have extensively been used for microencapsulation, with lower performance in islet transplant studies. Some of them are actually being rescued for application in new technologies for cell micro- and nano-encapsulation (see below). Because the majority of successful *in vivo* graft studies, including pilot clinical trials, have been conducted only with alginate-based microcapsules, these deserve most attention. Undoubtedly, the only polymer approved for fabricating microcapsules for human use remains alginate in its sodium salt [27]. Extracted from brown sea weeds, the alginic saccharydic polymers (guluronic and mannuronic acid block patterns) since the time of the first successful reports from Lim and Sun [28] have gained progressive popularity for the preparation of microcapsules for transplant purposes. A major issue in using the basic polymer has centered on the unavailability of ultrapurified alginate [29,30]. Since alginate is the major component of microcapsules, endotoxin and pyrogen-free criteria have to be thoroughly fulfilled in order to make this molecule suitable for clinical application [31].

2.3. Alginic polymer qualification for encapsulation for human use (University of Perugia technology)

The raw alginate powder available in the market ('pharmaceutical grade') must undergo an ultrapurification process. This eliminates several contaminants in order to obtain a final product that is chemically stable, endotoxin/pyrogen free and of low protein content that can be stored long-term with no loss of basic desirable physical–chemical properties (Table 3). This process can be performed in only a few selected centers worldwide, including the University of Perugia [27].

In our laboratory at the University of Perugia, we obtained a 'clinical-grade' basic alginate for capsule fabrication, using a special process to remove any lipopolysaccharidic contamination (USPatent 2010/0298262A1). The method is based on a multiple sequential filtration process through positively charged filters that allow ultrapurification of the powder and the resulting solution, and adjustments in pH and osmolality. Endotoxins are almost completely removed from the original preparation, whereas physical, chemical and molecular properties of the original product remain unchanged, as shown by ^{1}H and ^{14}C nuclear magnetic resonance spectra, examined before and after the polymeric purification procedure (Fig. 1A, B).

2.3.1. Protein content

Total protein content in the alginate samples was measured, according to guidelines from the Food and Drug Administration of the USA (FDA) by NanoOrange kit (Molecular Probes, Invitrogen, Milano, Italy). In particular, the 1.8% (w/v) alginate solution was diluted to a final concentration of 1% and assayed according to the manufacturer's instructions using a linear standard curve generated with bovine serum albumin.

2.3.2. Endotoxin content

The Endosafe (Charles River) was used to quantify the total endotoxin content of the alginates. In particular, 1.8% (w/v) alginate samples were diluted 10-fold in LAL reagent water (Charles River) and then fivefold in Endosafe-specific buffer (Charles River) following the manufacturer's instructions.

2.3.3. Heavy metal content

Heavy metal content in 1.8% (w/v) alginate solution was assessed according to standard procedures at the Central Core Clinical Chemistry Laboratory, Perugia University Hospital, Perugia, Italy.

2.4. Fabrication process

Standard 'medium-size' microcapsules were fabricated according to our published method [29], substantially modified from Sun and Lim

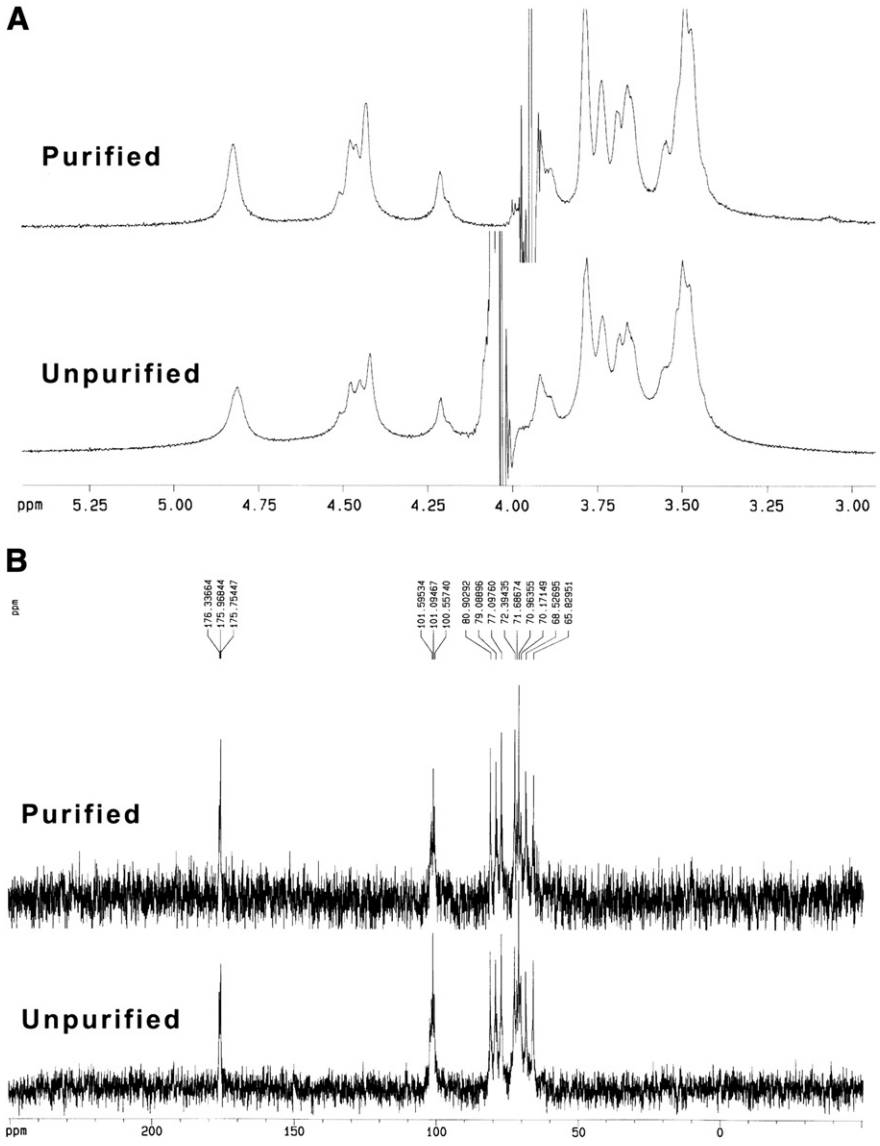

Fig. 1. (A) ^{1}H NMR spectra and (B) ^{14}C-NMR spectra of unpurified *versus* purified alginate solutions.

[28]. Briefly, ultrapure 1.6% Na-alginate solution was thoroughly mixed with islets to make a homogeneous alginate/islet cell suspension. The suspension was then extruded through a microdroplet generator (fabricated in our laboratory) in the form of microdroplets each containing one islet. The microdroplets were collected in a calcium chloride ($CaCl_2$) bath, and whereby immediately turned into gel microspheres. The gel beads were finally and sequentially coated with 0.12% poly-L-ornithine (PLO; Sigma) (single or double coating) and a final outer alginate layer, at appropriate stoichiometric molar ratios, to produce beads with biocompatibility and selective membrane permeability. The final microcapsules were uniform in size and shape measuring an average 500 μm in equatorial diameter, and containing an average of 1–2 islets per capsule. Such results were obtained after years of trials testing different islet cell pellet/alginate ratios. Now, in a standard islet encapsulation run, there are no free islets and few empty capsules.

2.5. Pre-clinical trials

A large number of pre-clinical studies where either allogeneic or xenogeneic (and autologous for control) islets were encapsulated and transplanted in non-immunosuppressed animal models of both artificially-induced and spontaneous diabetes – including rodents, canines and primates– have been produced by several centers, including our own, in the past three decades [32–37]. These started with the seminal work of Chang [38] and Sun [39]. These studies showed that, in the best experimental conditions, the encapsulated islet grafts indeed enabled reversal of hyperglycemia for variable periods of time, although diabetes correction was not indefinite. In the majority of these trials the capsules, mostly made of basic algin polymers, were implanted intraperitoneally or sometimes in pre-vascularized beds [40] showing variable problems. Many of these were related to either the average capsule size (600–800 μm) or the 'site of implant effect'. Taken together, these two factors often created unbalanced graft volume/site surface ratios, with consequential inadequate oxygen/nutrient supply, intracapsular islet death and capsular fibrotic overgrowth. Less tight capsule membranes, usually made of alginate gels with no amino-acid cation overcoating, were shown to be permeable to immunoglobulins Ig [41] and were apparently associated in mice with less performing results. Lessons from animal models taught us that sufficient islet mass (given the lower functional performance of encapsulated vs. free islets), capsule size and graft site effects were major issues to address, in order to upscale the encapsulated islet graft system for human application.

2.6. Clinical application of microencapsulated islets for patients with T1D

2.6.1. The University of Perugia experience

Upon completion of long-term pre-clinical trials of encapsulated allogeneic and xenogeneic islet transplantation (Tx) in either rodent or higher mammal models of diabetes, having assessed full safety and significant functional performance of our system, mainly due to the availability of our in-house system for alginate ultrapurification ('clinical grade'), we decided to embark on pilot human clinical trials. After a long series of audits, we were granted permission by the Italian Ministry of Health to initiate a closed, pilot phase-I clinical trial of microencapsulated human islet allo-Tx into non-immunosuppressed patients with long-standing T1D, on intensive insulin therapy regimens. So far we have completed four patients that have been followed-up throughout 5 years after the Tx.

The primary goal of this study was to determine long-term safety of the encapsulated human islet (HI)Tx. The following parameters were examined: (1) Tx-related adverse reactions; (2) Tx-directed immune reactivity; and (3) host sensitization to grafted encapsulated islet cell antigens.

Only excellent human islet preparations (Fig. 2A, B) that were proven morphologically intact and functional, upon static incubation with glucose, were considered for donor islets, and underwent microencapsulation prior to Tx.

We also examined the following metabolic parameters: (1) changes in exogenous insulin consumption; (2) levels of prior negative serum C-peptide responsiveness; (3) changes in severe nocturnal hypoglycemia, as defined by BG,40 mg/dL (patients 1 and 2); and (4) changes in HbA1c plasma levels.

All four patients were grafted intraperitoneally, under local anesthesia and ultrasound echography guidance; except for one case who received three subsequent grafts, the last delivered under general anesthesia, by laparoscopic surgery. The aim, in this last instance, was to deliver the encapsulated islets in areas that would be better vascularized (i.e. mesentery), unlike the great peritoneal cavity. In all cases we observed neither adverse reactions to the grafting procedure, nor evidence of immune sensitization. In terms of metabolic outcome, all patients showed decline of exogenous daily insulin consumption – which nearly halved – except one case that temporarily suspended the insulin injections. C-peptide levels were detected in all cases and lasted for 100–480 days, reaching peaks of 1.8 ng/mL in patient #4 in basal and after stimulation. As a unique finding, no anti-MHC class I–II, anti-GAD65 antibodies or islet cell antibodies were detected in any of the transplanted patients throughout the five years of post-Tx follow-up [42,43]. Hence, microcapsules prevented any detection of foreign antigens by the host, despite the lack of immunosuppressive treatment. In our opinion, this was the most important finding of the study. Obviously, the partial and transient metabolic benefits obtained by the treatment reflect limitations of this microcapsule generation, particularly concerning their size with regard to the Tx site. We maintain that smaller microcapsules could permit access to Tx sites that are possibly associated with better biochemical exchange — thereby complying, in a more efficient fashion, with metabolic requirements of the patients with T1D. No adverse effects were reported except patient #2, who for near to five years post-Tx complained of superficial abdominal discomfort. The pain was associated with a small mass palpable on the anterior abdominal region. Upon ultrasound imaging, the patient underwent minor surgery, under local anesthesia, that was associated with finding and removing a cyst-like formation, measuring 3 cm, situated beneath the fascia of the anterior rectus muscle. Evidently part of the microcapsule graft had not been delivered intraperitoneally, but rather more superficially. After five years the cyst contained capsules that appeared mostly intact (Fig. 2C) although the majority contained only necrotic debris of what were once viable human islets. At that time the patient had returned to his, prior to Tx, full insulin schedule.

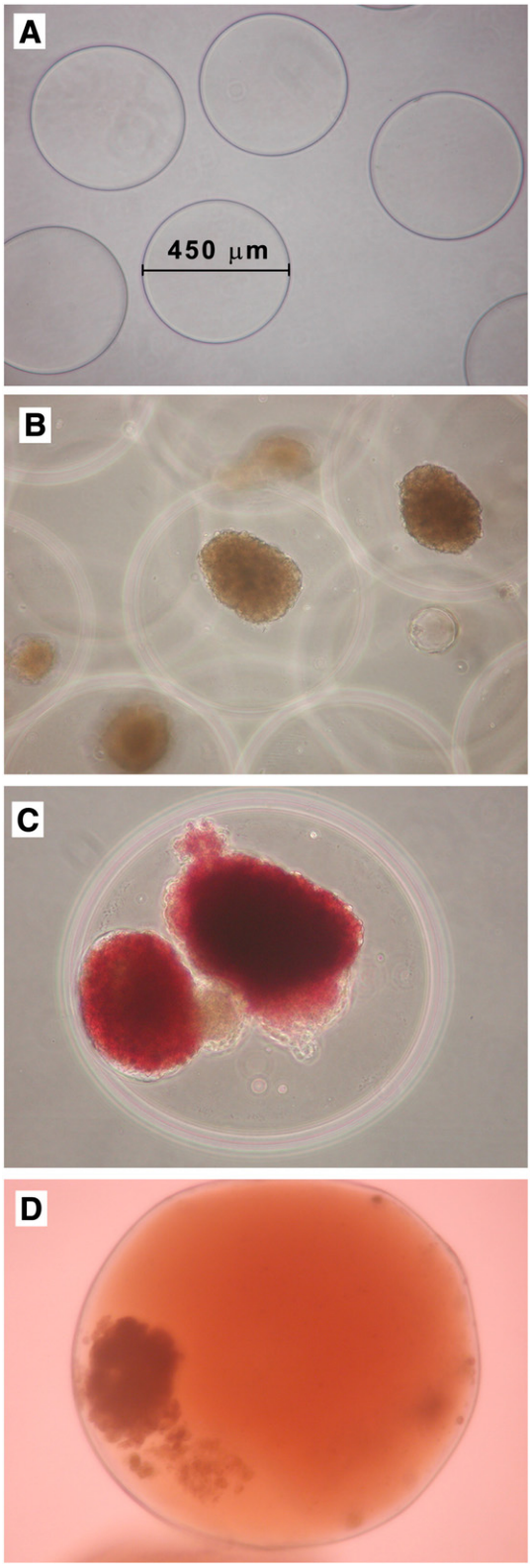

Fig. 2. (A) Empty capsules at the end of the procedure. (B) Human islet containing microcapsules. (C) Encapsulated human islets retrieved from patient #1, 5 years after TX.

2.6.2. The University of New South Wales, Australia experience

A different phase I clinical study was conducted with barium alginate microcapsules by Tuch and fellow investigators [44]. In this study, four patients with T1D and no detectable C-peptide were infused with human islets encapsulated within barium alginate microcapsules

intraperitoneally without general immunosuppression. C-peptide was detected on day 1 post-transplantation, and BG and insulin requirements decreased, albeit transiently. C-peptide was again undetectable by 1–4 weeks. In a multi-islet recipient, C-peptide was detected at 6 weeks after the third infusion and remained detectable throughout 2.5 years. Neither insulin requirement nor glycemic control was significantly affected by the release of this small amount of insulin. To better understand what occurred in the transplanted capsules, a laparoscopy was performed in the recipient of the four encapsulated islet infusions at 16 months after the first procedure. Large numbers of capsules were found scattered throughout the peritoneal cavity in clusters attached to the parietal peritoneum, spleen, omentum and kidney. A biopsy showed that the capsules were intact and surrounded by fibrous tissue containing thin-walled capillaries with a mild histiocytic response. The encapsulated islets were necrotic. The loss of graft function was probably due to either ischemic necrosis or an inflammatory process, possibly initiated by fibrinogen adhering to the capsule's surface.

2.6.3. The Living Cell Technologies (LCT) experience

LCT started collaborative work with the University of Perugia team in 1999, with exchanges of the respective products (encapsulation technology from the University of Perugia and post-natal pig islets from LCT). From this alliance several experimental trials in either rodents or primates with spontaneous or artificially-induced diabetes were set up, creating the ground for subsequent initiation of pilot phase I/II clinical trials of microencapsulated porcine islet xenografts into patients with type 1 (or anyway insulin-dependent diabetes). Two recent studies describing transplantation of microencapsulated neonatal pig islets in an alginate matrix confirmed their biocompatibility in non-diabetic monkeys as well as their capacity to partially regulate diabetes [45,46]. LCT showed that porcine islet cells had survived in a human patient 10 years after transplant of pig islet cells [47]. These findings demonstrated the long-term safety, viability and function — although uncoupled with systemic effects of encapsulated porcine islets in a human patient, with no general immunosuppression. Two phase I trials have shown that intraperitoneally infused microencapsulated human islets can be considered safe for up to 3 years [48]. Although insulin independence was not achieved, glycemic control was improved, with a reduction in insulin daily requirement. In 2007, LCT launched a phase I/IIa study in Moscow of encapsulated neonatal insulin-producing porcine pancreatic islet cells (commercially called DIABECELL®). Seven patients with insulin-dependent diabetes received 1–3 implants of DIABECELL® (5000 and 10,000 IEQ/kg), with none showing marked adverse events at 18–96 weeks after transplantation.

All recipients showed improvements in diabetes control, with lower glycated hemoglobin (% HbA1c) concentrations. Following the successful completion of this phase I/IIa clinical trial in Russia, LCT launched phase IIb clinical trials, which are currently underway in New Zealand and Argentina.

Concerning the Argentinian trial, a press release was issued by LCT at the end of 2012 reporting the following: *For Press Release – 22 November 2012 Sydney, Australia and Auckland, New Zealand* – "Living Cell Technologies Limited (ASX: LCT; OTCQX: LVCLY) today announced the results of an interim analysis of its Argentinian Phase I/IIa clinical trial for DIABECELL, a breakthrough treatment for people with unstable type 1 diabetes."

The results clearly demonstrated a clinically significant reduction in HbA1c, insulin dose and unaware hypoglycemia, with greater benefit seen in the patient group receiving the higher dose of DIABECELL®.

These trials, beyond press release statements, cannot be considered true 'breakthroughs', since no exogenous insulin supplementation was discontinued, and the achieved metabolic results were minor and not different from what was obtained elsewhere. However, an important 'take home message' from these pilot clinical trials is that a major achievement, namely safety of an encapsulated islet xenograft procedure in humans, has been accomplished. It is expected that a sufficient islet cell mass per recipient, coupled with better selection of the capsules' site of implanting, as well as improvements in the membranes' size/formulation will definitely help in achieving a proficient metabolic outcome.

3. Encapsulated islet allografts in humans with no systemic immunosuppression: advances and critique

Bringing the alginate-based islet cell encapsulation system into human trials has coincided with a major achievement, after over two decades of experimental animal studies. These studies, more convincingly in some centers, have preliminarily but clearly shown how important are the alginate composites, in terms of the selected chemistries and use of amino-acid polycation outer coating: some capsules' fabrication principles cannot be ignored without compromising strength of the graft outcome. Another very recent report confirmed this positive trend of encapsulated human islets for T1D [49].

The only well-documented study so far conducted at the University of Perugia incorporated a careful 5 years of post-TX follow-up, with the following important results:

1. No host immune sensitization towards the encapsulated islet allografts occurred. This was clearly documented by the absence of anti-MHC class I–II, as well as anti-GAD65 and ICA AAb throughout the 5 years of post-TX follow-up;
2. No significant adverse effects have occurred in any of the transplanted patients, except for one patient where the encapsulated islet suspension was partially injected into an unwanted site (fascia of the rectus anterior muscle) and not intraperitoneally as scheduled;
3. Graft function, as demonstrated by C-peptide serum levels lasted for throughout 400 days of post-TX, in conjunction with a significant decrease of HbA1c levels as well as hypoglycemia unawareness episodes. These results were also deemed to be metabolically relevant, in terms of overall glucose control in T1D patients, by those authors that performed non-encapsulated islet allografts into pharmacologically immunosuppressed patients with T1D.

In our studies we clearly proved the safety of the procedure, as well as partial but evident graft function. Moreover tolerance of the grafts, shown by the absence of recipient sensitization, was granted by the ultrapure alginate blend ad hoc complexed with PLO.

Other studies, employing different alginate blends (mainly Ba alginates salts) with no polyamino-acid coating, have demonstrated product safety due to the simplicity of the grafting procedures. Nonetheless, these did not achieve immunoisolation due to the loose membrane's molecular weight cut-off, while biocompatibility was variable, sometimes showing intense peri-capsular fibrotic tissue overgrowth. Only in immunodeficient mice was biocompatibility evident [49].

Hence the lack of reproducibility of the exhibited results from different centers may reflect major differences in algin polymers' purity and basic chemistry. Many studies have clearly indicated alginate purity as an indispensable feature for a successful outcome of encapsulated islet grafts [29–31]. Likewise, imperfect capsule morphology leading to abnormal shape, 'polydispersity' and islet cell protrusion through the outer capsule membrane are well known risks for macrophage signaling [36,37] and encapsulated islet-directed immune targeting. On the contrary, rigorously formulated, regularly and smoothly shaped microcapsules favor the grafted islets' immunoisolation, biological acceptance and positive functional graft outcome [50].

The lessons learned from these preliminary encapsulated allogeneic/xenogeneic islet grafts in patients with T1D worldwide indicate that if all the state-of-the-art technical principles for islet microencapsulation are complied with, then positive results in terms of safety and ease of clinical application may be obtained. However, technical pitfalls still hamper a clear successful functional outcome of these grafts, with some issues deserving special attention:

1. Capsule size. Final graft volume per patient is still excessive, and smaller capsules would functionally perform better.
2. Site of implant effect. The peritoneal cavity, if easily accessible for the graft with no traumatizing procedures, is not ideal in terms of both oxygen/nutrient supply and pharmacokinetics of the secreted insulin. Smaller capsules could be grafted elsewhere.
3. Low functional islet mass loaded in the capsules is associated with insufficient insulin output.

This latter problem is less stringent with xenogeneic porcine islets but still a pending problem, as illustrated above.

4. Future directions

New macrodevices, as far as their immunoisolation, biocompatibility and the embodied islet survival and functional performance are actually pushing forward, approaching human application, but are not discussed in this chapter. New strategies for microcapsules are being pursued in order to counteract weaknesses associated with the conventional products so far illustrated.

4.1. Generation of smaller microcapsules

The focus for the next generation of microcapsules should be on consistently reducing their size to achieve a 'conformal' configuration that, while hosting islets or insulin-producing cell clusters within an immunoprotective and biocompatible shield, would also enable graft lodging in smaller areas, possibly not the peritoneal cavity. Several conformal capsules are under preparation at this juncture following different fabrication procedures (see below).

4.2. Two aqueous-phase emulsification

4.2.1. Alginates

Emulsification has been used to generate a conformal coating surrounding islets or cell aggregates. Calafiore et al. placed islets into a two aqueous-phase alginate/PEG/Ficoll emulsion, whereby alginate-containing Ficoll droplets suspended in a continuous PEG phase coalesced on individual islet surface, engulfing each islet within an alginate droplet. This early product was subsequently treated with $CaCl_2$ and coated with a PLO/alginate bilayer. Reagent stoichiometric molar ratios are extremely important in order to avoid membrane shrinkage and rupture, with consequential loss of immunoisolation and biocompatibility properties of these minicoatings. This conformal barrier does prevent direct contact between islets and antibodies, and does not impair insulin secretion in response to glucose stimulation. In the University of Perugia laboratory this emulsification process was recently optimized to minimize the incidence of incomplete or uneven coating, by overlaying the cell clusters within thin (20–25 μm) alginate films [35]. Selective withdrawal of one fluid through a second immiscible phase has recently been used to encapsulate pancreatic islets [51]. At this preliminary stage of development it is premature to speculate on the effective extent of immunoprotection afforded by these coatings.

4.2.2. PEG

Islets suspended in PEG-diacrylate were layered onto a denser, immiscible oil phase, and fluid was withdrawn through a tube placed immediately below the interface. PEG-diacrylate was subsequently photocross linked, resulting in about 10-μm-thick coatings independent of size of the encapsulated islets. The investigators found it necessary to repeat this process to prevent coating defects, ultimately generating coatings of about 20 μm thickness. In principle, sequential coating may allow for the generation of composite coatings, with possible intralayer embodiment of anti-inflammatory or proangiogenic molecules, with improvement of the membrane's performance. Two-layer conformal coatings were found to inhibit the transport of a 140-kDa macromolecule and enabled normal dynamic insulin secretion in response to glucose stimulation [52]. Despite these promising results, about 75% of the islets were lost during the coating process, while the method's scalability remains an open issue.

4.2.3. Interfacial polymerization

Strategies to fabricate conformal coatings have also tried to use the islet surface as a template upon which coatings may be grown or chemically deposited. Cruise et al. generated about 35–50-μm thick PEG-diacrylate coatings on both porcine [53] and human [54] islets through a process of interfacial polymerization. Eosin Y, a photoinitiator, is non-specifically adsorbed to the islet surface, and islets are placed in a solution containing PEG-diacrylate and triethanolamine. Upon illumination with light, eosin Y is excited and this initiates the free-radical polymerization of PEG-diacrylate at the islet–macromer interface. Through parametric optimization of key process variables, greater than 90% islet viability and encapsulation efficiency have been reported, and conformally coated islets were compared to uncoated islets during *in vitro* glucose perfusion, and *in vivo* peritoneal glucose tolerance tests. Pre-clinical trials in diabetic cynomolgus monkeys and baboons demonstrated the function of subcutaneously transplanted conformally coated islets for up to 20 months, despite discontinuation of low-dose immunosuppression, 1 month post-transplant [50], although the long-term outcome of this approach is unknown.

4.2.4. Layer-by-layer assembly of polyelectrolyte multilayer

Layer-by-layer assembly of polyelectrolyte multilayer (PEM) films represents a bottom-up approach for re-engineering the molecular landscape of cell surfaces with spatially continuous and molecularly uniform ultrathin films. Several groups are currently engaged in this innovative research. Starting from his original agarose recipe, Teramura et al. [54] further engineered the capsular membrane using maleimide-conjugated PEG-lipids, 5000-molecular weight PEG, with subsequent polyvinyl alcohol bound to PEG. The ultrathin formed membrane seems to be counterbalanced by complexity of the fabrication process and questionable long-term stability. Moreover, only *in vitro* static incubation studies have been performed with these membranes. A subset of cationic copolymers undergoing conformational changes, which mitigate membrane disruption and facilitate the deposition of PEMs on cell surfaces – subject to alterations in composition, reactivity, thickness, and mechanical properties – are also being developed [55,56]. The first successful *in vivo* application of PEM-engineered cells, which maintained viability and function upon transplantation and were used as carriers for *in vivo* delivery of PEMs containing biomolecular payloads, has been reported. This new class of polymeric films and the design strategies developed therein may consolidate a technology for cell transplantation and other therapies based on engineered cells [55]. It had been previously demonstrated that PEG chains could reduce the cytotoxicity of polycations [22,57].

Through appropriate control of structural variables, pegylated poly-L-lysine PLL-g-PEG copolymers can be rendered cytocompatible while simultaneously facilitating layer-by-layer self-assembly of PEM films, directly on the surface of cells composing a pancreatic islet. Alginate was chosen as the polyanionic species. As a demonstration of this concept, cytocompatible PLL-g-PEG copolymers bearing biotin- and azide-functionalized PEG grafts were used as a terminal layer in film assembly to confer biological specificity and chemical reactivity, respectively. Films assembled with biotin-functionalized Alg PLL-g-PEG copolymers specifically bound Cy3-labeled streptavidin through biorecognition, providing an easy approach to incorporating biotinylated molecules or streptavidin drug conjugates into cell surface-supported films. Importantly, the viability of islets coated with eight bilayer P12PnDc]/alginate PEM films was statistically indistinguishable ($P > 0.01$) from untreated controls both immediately and 18–24 h after the film assembly [57]. Moreover, the functional capacity of islets to release insulin in a

glucose-responsive manner was not adversely influenced by film formation, as islets engineered with PEMs secreted statistically similar amounts of insulin at both basal and high glucose concentrations compared with untreated controls. Unfortunately, *in vivo* graft studies in diabetic animals have shown only partial and short-term evidence of function [58].

5. Alternative sites of implant

It is plausible that should the new conformal capsules, whether composed of alginates or alternative biopolymers according to different fabrication procedures, be proven to retain immunoisolation and biocompatibility properties then these new capsules would offer the opportunity to consider alternative sites of implanting. One possibility would be to prepare pre-vascularized, artificial polymeric beds situated either in the subcutaneous space or in the mesenteric area where the small islet/insulin producing cells' conformal coatings could be deposited. The macro-beds could be suitable for capsule filling, retrieval or replenishment procedures, should the β-cell function be exhausted over time.

6. Conclusions

There are few doubts about the fact that during recent years microencapsulation technologies have substantially progressed, thus making their application to humans possible after many years of experimental animal studies only. While human application has been associated with positive outcomes in terms of the capsules' material qualification that have resulted in evident safety of the grafted product, several gaps need to be filled before encapsulated islets and/or insulin-producing cells with functional performance of sufficient degree of confidence. Towards this end, several centers are intensively working on capsule engineering to render them suitable for graft in selected areas closer to metabolic requirements of a diabetic organism.

Should this goal be reached in the near future, the resulting artificial biohybrid pancreas would certainly displace conventional naked islet transplantation, under general recipient immunosuppression, for the final possible cure of T1D.

Acknowledgments

The kind assistance of Dr. Pia Montanucci is gratefully acknowledged.

References

[1] S.F. Hansson, S. Korsgren, F. Pontén, O. Korsgren, Enteroviruses and the pathogenesis of type 1 diabetes revisited: crossreactivity of enterovirus capsid protein (VP1) antibodies with human mitochondrial proteins, 23rd Joint AIDPIT and EPITA Winter Symposium 27th–29th January 2013, 2013.
[2] W.T. Cefalu, Diabetes Care: "State of the Union", Diabetes Care 36 (2013) 1–3.
[3] F.B. Barton, M.R. Rickels, R. Alejandro, B.J. Hering, S. Wease, B. Naziruddin, J. Oberholzer, J.S. Odorico, M.R. Garfinkel, M. Levy, F. Pattou, T. Berney, A. Secchi, S. Messinger, P.A. Senior, P. Maffi, A. Posselt, P.G. Stock, D.B. Kaufman, X. Luo, F. Kandeel, E. Cagliero, N.A. Turgeon, P. Witkowski, A. Naji, P.J. O'Connell, C. Greenbaum, Y.C. Kudva, K.L. Brayman, M.J. Aull, C. Larsen, T.W. Kay, L.A. Fernandez, M.C. Vantyghem, M. Bellin, A.M. Shapiro, Improvement in outcomes of clinical islet transplantation: 1999–2010, Diabetes Care 35 (2012) 1436–1445.
[4] M.D. Bellin, M.L. Freeman, S.J. Schwarzenberg, T.B. Dunn, G.J. Beilman, S.M. Vickers, S. Chinnakotla, A.N. Balamurugan, B.J. Hering, D.M. Radosevich, A. Moran, D.E. Sutherland, Quality of life improves for pediatric patients after total pancreatectomy and islet autotransplant for chronic pancreatitis, Clin. Gastroenterol. Hepatol. 9 (2011) 793–799.
[5] T. Tharavanij, A. Betancourt, S. Messinger, P. Cure, C.B. Leitao, D.A. Baidal, T. Froud, C. Ricordi, R. Alejandro, Improved long-term health-related quality of life after islet transplantation, Transplantation 86 (2008) 1161–1167.
[6] P. Fiorina, C. Gremizzi, P. Maffi, R. Caldara, D. Tavano, L. Monti, C. Socci, F. Folli, F. Fazio, E. Astorri, A. Del Maschio, A. Secchi, Islet transplantation is associated with an improvement of cardiovascular function in type 1 diabetic kidney transplant patients, Diabetes Care 28 (2005) 1358–1365.
[7] D.M. Thompson, M. Meloche, Z. Ao, B. Paty, P. Keown, R.J. Shapiro, S. Ho, D. Worsley, M. Fung, G. Meneilly, I. Begg, M. Al Mehthel, J. Kondi, C. Harris, B. Fensom, S.E. Kozak, S.O. Tong, M. Trinh, G.L. Warnock, Reduced progression of diabetic microvascular complications with islet cell transplantation compared with intensive medical therapy, Transplantation 91 (2011) 373–378.
[8] C.B. Leitão, P. Cure, S. Messinger, A. Pileggi, O. Lenz, T. Froud, R.N. Faradji, G. Selvaggi, W. Kupin, C. Ricordi, R. Alejandro, Stable renal function after islet transplantation: importance of patient selection and aggressive clinical management, Transplantation 87 (2009) 681–688.
[9] U. Del Carro, P. Fiorina, S. Amadio, L. De Toni Franceschini, A. Petrelli, S. Menini, F. Martinelli Boneschi, S. Ferrari, G. Pugliese, P. Maffi, G. Comi, A. Secchi, Evaluation of polyneuropathy markers in type 1 diabetic kidney transplant patients and effects of islet transplantation: neurophysiological and skin biopsy longitudinal analysis, Diabetes Care 30 (2007) 3063–3069.
[10] T.C. Lee, N.R. Barshes, C.A. O'Mahony, L. Nguyen, F.C. Brunicardi, C. Ricordi, R. Alejandro, A.P. Schock, A. Mote, J.A. Goss, The effect of pancreatic islet transplantation on progression of diabetic retinopathy and neuropathy, Transplant. Proc. 37 (2005) 2263–2265.
[11] G.L. Warnock, D.M. Thompson, R.M. Meloche, R.J. Shapiro, Z. Ao, P. Keown, J.D. Johnson, C.B. Verchere, N. Partovi, I.S. Begg, M. Fung, S.E. Kozak, S.O. Tong, K.M. Alghofaili, C. Harris, A multi-year analysis of islet transplantation compared with intensive medical therapy on progression of complications in type 1 diabetes, Transplantation 86 (2008) 1762–1766.
[12] D.M. Thompson, I.S. Begg, C. Harris, Z. Ao, M.A. Fung, R.M. Meloche, P. Keown, G.S. Meneilly, R.J. Shapiro, S. Ho, K.G. Dawson, A.I.K. Ghofaili, L. Al Riyami, M. Al Mehthel, S.E. Kozak, S.O. Tong, G.L. Warnock, Reduced progression of diabetic retinopathy after islet cell transplantation compared with intensive medical therapy, Transplantation 85 (2008) 1400–1405.
[13] P. Maffi, F. Bertuzzi, F. De Taddeo, P. Magistretti, R. Nano, P. Fiorina, A. Caumo, P. Pozzi, C. Socci, M. Venturini, A. Del Maschio, A. Secchi, Kidney function after islet transplant alone in type 1 diabetes: impact of immunosuppressive therapy on progression of diabetic nephropathy, Diabetes Care 30 (2007) 1150–1155.
[14] P. Cure, A. Pileggi, T. Froud, S. Messinger, R.N. Faradji, D.A. Baidal, R. Cardani, A. Curry, R. Poggioli, A. Pugliese, A. Betancourt, V. Esquenazi, G. Ciancio, G. Selvaggi, G.W. Burke, C. Ricordi, R. Alejandro, Improved metabolic control and quality of life in seven patients with type 1 diabetes following islet after kidney transplantation, Transplantation 85 (2008) 801–812.
[15] Y. Yasunami, S. Kojo, H. Kitamura, A. Toyofuku, M. Satoh, M. Nakano, K. Nabeyama, Y. Nakamura, N. Matsuoka, S. Ikeda, M. Tanaka, J. Ono, N. Nagata, O. Ohara, M. Taniguchi, Valpha14 NK T cell-triggered IFN-gamma production by Gr-1+CD11b+cells mediates early graft loss of syngeneic transplanted islets, J. Exp. Med. 202 (2005) 913–918.
[16] O. Korsgren, T. Lundgren, M. Felldin, A. Foss, B. Isaksson, J. Permert, N.H. Persson, E. Rafael, M. Rydén, K. Salmela, A. Tibell, G. Tufveson, B. Nilsson, Optimising islet engraftment is critical for successful clinical islet transplantation, Diabetologia 51 (2008) 227–232.
[17] N.M. Desai, J.A. Goss, S. Deng, B.A. Wolf, E. Markmann, M. Palanjian, A.P. Shock, S. Feliciano, F.C. Brunicardi, C.F. Barker, A. Naji, J.F. Markmann, Elevated portal vein drug levels of sirolimus and tacrolimus in islet transplant recipients: local immunosuppression or islet toxicity? Transplantation 76 (2003) 1623–1625.
[18] P. Montanucci, I. Pennoni, T. Pescara, P. Blasi, G. Bistoni, G. Basta, R. Calafiore, The functional performance of microencapsulated human pancreatic islet-derived precursor cells, Biomaterials 32 (2011) 9254–9262.
[19] M. Chayosumrit, B. Tuch, K. Sidhu, Alginate microcapsule for propagation and directed differentiation of hESCs to definitive endoderm, Biomaterials 31 (2010) 505–514.
[20] G. Karoubi, M.L. Ormiston, D.J. Stewart, D.W. Courtman, Single-cell hydrogel encapsulation for enhanced survival of human marrow stromal cells, Biomaterials 30 (2009) 5445–5455.
[21] T. Yasuhara, I. Date, Intracerebral transplantation of genetically engineered cells for Parkinson's disease: toward clinical application, Cell Transplant. 16 (2007) 125–132.
[22] K.K. Hall, K.M. Gattás-Asfura, C.L. Stabler, Microencapsulation of islets within alginate/poly(ethylene glycol) gels cross-linked via Staudinger ligation, Acta Biomater. 7 (2011) 614–624.
[23] S. Kizilel, A. Scavone, X. Liu, J.M. Nothias, D. Ostrega, P. Witkowski, M. Millis, Encapsulation of pancreatic islets within nano-thin functional polyethylene glycol coatings for enhanced insulin secretion, Tissue Eng. 16 (2010) 2217–2228.
[24] T. Kobayashi, Y. Aomatsu, H. Iwata, Indefinite islet protection from autoimmune destruction in nonobese diabetic mice by agarose microencapsulation without immunosuppression, Transplantation 75 (2003) 619–625.
[25] J.J. Vallbacka, M.V. Sefton, Vascularization and improved in vivo survival of VEGF-secreting cells microencapsulated in HEMA-MMA, Tissue Eng. 13 (2007) 2259–2269.
[26] O.F. Khan, M.V. Sefton, Patterning collagen/poloxamine-methacrylate hydrogels for tissue-engineering-inspired microfluidic and laser lithography applications, J. Biomater. Sci. Polym. Ed. 22 (2011) 2499–2514.
[27] P. de Vos, M.M. Faas, B. Strand, R. Calafiore, Alginate-based microcapsules for immunoisolation of pancreatic islets, Biomaterials 27 (2006) 5603–5617.
[28] F. Lim, A.M. Sun, Microencapsulated islets as bioartificial endocrine pancreas, Science 210 (1980) 908–910.
[29] R. Calafiore, G. Basta, Artificial pancreas to treat type 1 diabetes mellitus, Meth. Mol. Med. 140 (2007) 197–236.
[30] G. Langlois, J. Dusseault, S. Bilodeau, S.K. Tam, D. Magassouba, J.P. Hallé, Direct effect of alginate purification on the survival of islets immobilized in alginate-based microcapsules, Acta Biomater. 5 (2009) 3433–3440.
[31] J. Dusseault, S.K. Tam, M. Ménard, S. Polizu, G. Jourdan, L. Yahia, J.P. Hallé, Evaluation of alginate purification methods: effect on polyphenol, endotoxin, and protein contamination, J. Biomed. Mater. Res. A 76 (2006) 243–251.

[32] A. Omer, V. Duvivier-Kali, J. Fernandes, V. Tchipashvili, C.K. Colton, G.C. Weir, Long-term normoglycemia in rats receiving transplants with encapsulated islets, Transplantation 79 (2005) 52–58.
[33] T. Kin, H. Iwata, Y. Aomatsu, T. Ohyama, H. Kanehiro, M. Hisanaga, Y. Nakajima, Xenotransplantation of pig islets in diabetic dogs with use of a microcapsule composed of agarose and polystyrene sulfonic acid mixed gel, Pancreas 25 (2002) 94–100.
[34] D. Dufrane, R.M. Goebbels, A. Saliez, Y. Guiot, P. Gianello, Six month survival of microencapsulated pig islets and alginate biocompatibility in primates: proof of concept, Transplantation 81 (2006) 1345–1353.
[35] R. Calafiore, G. Basta, G. Luca, C. Boselli, A. Bufalari, A. Bufalari, M.P. Cassarani, G.M. Giustozzi, P. Brunetti, Transplantation of pancreatic islets contained in minimal volume microcapsules in diabetic high mammalians, Ann. N. Y. Acad. Sci. 875 (1999) 219–232.
[36] P. de Vos, I. Smedema, H. van Goor, H. Moes, J. van Zanten, S. Netters, L.F. de Leij, A. de Haan, B.J. de Haan, Association between macrophage activation and function of micro-encapsulated rat islets, Diabetologia 46 (2003) 666–673.
[37] P. de Vos, A.F. Hamel, K. Tatarkiewicz, Considerations for successful transplantation of encapsulated pancreatic islets, Diabetologia 45 (2002) 159–173.
[38] T.M. Chang, Semipermeable microcapsules, Science 146 (1964) 524–525.
[39] Y. Sun, X. Ma, D. Zhou, I. Vacek, A.M. Sun, Normalization of diabetes in spontaneously diabetic cynomologus monkeys by xenografts of microencapsulated porcine islets without immunosuppression, J. Clin. Invest. 98 (1996) 1417–1422.
[40] P. de Vos, J.L. Hillebrands, B.J. De Haan, J.H. Strubbe, R. Van Schilfgaarde, An artificial transplantation site for pancreatic islets, Transplant. Proc. 30 (1998) 484.
[41] Y.A. Mørch, I. Donati, B.L. Strand, G. Skjåk-Braek, Effect of Ca2, Ba2, and Sr2 on alginate microbeads, Biomacromolecules 7 (2006) 1471–1480.
[42] R. Calafiore, G. Basta, G. Luca, A. Lemmi, M.P. Montanucci, G. Calabrese, L. Racanicchi, F. Mancuso, P. Brunetti, Microencapsulated pancreatic islet allografts into nonimmunosuppressed patients with type 1 diabetes: first two cases, Diabetes Care 29 (2006) 137–138.
[43] G. Basta, P. Montanucci, G. Luca, C. Boselli, G. Noya, B. Barbaro, M. Qi, K.P. Kinzer, J. Oberholzer, R. Calafiore, Long-term metabolic and immunological follow-up of nonimmunosuppressed patients with type 1 diabetes treated with microencapsulated islet allografts: four cases, Diabetes Care 34 (2011) 2406–2409.
[44] B.E. Tuch, G.W. Keogh, L.J. Williams, W. Wu, J.L. Foster, V. Vaithilingam, R. Philips, Safety and viability of microencapsulated human islets transplanted into diabetic humans, Diabetes Care 32 (2009) 1887–1889.
[45] C.G. Thanos, R. Calafiore, G. Basta, B.E. Bintz, W.J. Bell, J. Hudak, A. Vasconcellos, P. Schneider, S.J. Skinner, M. Geaney, P. Tan, R.B. Elliot, M. Tatnell, L. Escobar, H. Qian, E. Mathiowitz, D.F. Emerich, Formulating the alginate-polyornithine biocapsule for prolonged stability: evaluation of composition and manufacturing technique, J. Biomed. Mater. Res. A 83 (2007) 216–224.
[46] R.B. Elliott, L. Escobar, P.L. Tan, O. Garkavenko, R. Calafiore, P. Basta, A.V. Vasconcellos, D.F. Emerich, C. Thanos, C. Bambra, Intraperitoneal alginate-encapsulated neonatal porcine islets in a placebo-controlled study with 16 diabetic cynomolgus primates, Transplant. Proc. 37 (2005) 3505–3508.
[47] R.B. Elliott, L. Escobar, P.L. Tan, M. Muzina, S. Zwain, C. Buchanan, Live encapsulated porcine islets from a type 1 diabetic patient 9.5 yr after xenotransplantation, Xenotransplantation 14 (2007) 157–161.
[48] R.B. Elliott, Communication to 23th World Congress of IPITA. Prague, Czech Republic, 2011.
[49] D. Jacobs-Tulleneers-Thevissen, M. Chintinne, Z. Ling, P. Gillard, L. Schoonjans, G. Delvaux, B.L. Strand, F. Gorus, B. Keymeulen, D. Pipeleers, Beta Cell Therapy Consortium EU-FP7. Sustained function of alginate-encapsulated human islet cell implants in the peritoneal cavity of mice leading to a pilot study in a type 1 diabetic patient, Diabetologia 56 (2013) 1605–1614.
[50] G. Basta, P. Sarchielli, G. Luca, L. Racanicchi, C. Nastruzzi, L. Guido, F. Mancuso, G. Macchiarulo, G. Calabrese, P. Brunetti, R. Calafiore, Optimized parameters for microencapsulation of pancreatic islet cells: an in vitro study clueing on islet graft immunoprotection in type 1 diabetes mellitus, Transpl. Immunol. 13 (2004) 289–296.
[51] A. Leung, Y. Ramaswamy, P. Munro, G. Lawrie, L. Nielsen, M. Trau, Emulsion strategies in the microencapsulation of cells: pathways to thin coherent membranes, Biotechnol. Bioeng. 92 (2005) 45–53.
[52] D.Y. Lee, S.J. Park, J.H. Nam, Y. Byun, A new strategy toward improving immunoprotection in cell therapy for diabetes mellitus: long functioning PEGylated islets in vivo, Tissue Eng. 12 (2006) 615–623.
[53] G.M. Cruise, O.D. Hegre, D.S. Scharp, J.A. Hubbell, A sensitivity study of the key parameters in the interfacial photopolymerization of poly (ethylene glycol) diacrylate upon porcine islets, Biotechnol. Bioeng. 57 (1998) 655–665.
[54] Y. Teramura, Y. Kaneda, H. Iwata, Islet-encapsulation in ultra-thin layer-by-layer membranes of poly(vinyl alcohol) anchored to poly (ethylene glycol)-lipids in the cell membrane, Biomaterials 28 (2007) 4818–4825.
[55] J.T. Wilson, W. Cui, E.L. Chaikof, Layer-by-layer assembly of a conformal nanothin PEG coating for intraportal islet transplantation, Nano Lett. 8 (2008) 1940–1948.
[56] D. Xie, C.A. Smyth, C. Eckstein, G. Bilbao, J. Mays, D.E. Eckhoff, J.L. Contreras, Cytoprotection of PEG modified adult porcine pancreatic islets for improved, xenotransplantation, Biomaterials 26 (2005) 403–412.
[57] J.T. Wilson, W. Cui, V. Kozlovskaya, E. Kharlampieva, D. Pan, Z. Qu, V.R. Krishnamurthy, J. Mets, V. Kumar, J. Wen, Y. Song, V.V. Tsukruk, E.L. Chaikof, Cell surface engineering with polyelectrolyte multilayer thin films, J. Am. Chem. Soc. 133 (2011) 7054–7064.
[58] D.Y. Lee, J.H. Nam, Y. Byun, Effect of polyethylene glycol grafted onto islet capsules on prevention of splenocyte and cytokine attacks, J. Biomater. Sci. Polym. Ed. 15 (2004) 753–766.