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Therapeutic cell encapsulation techniques and applications in diabetes ☆

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ABSTRACT

The encapsulation of therapeutic cells permits the implantation of allogeneic and xenogeneic cells for the regulation of certain physiological processes damaged by the death or senescence of host tissues. The encapsulation of pancreatic cells for the treatment of diabetes is emphasized; however, many of the techniques are applicable to a wide array of mammalian cell applications. The summary of both established and novel encapsulation techniques, clinical trials, and commercial product developments highlights the metered but steady pace of therapeutic cell encapsulation towards implementation.

Contents

1. Introduction

The term 'bioencapsulation' generally refers to the entrapment or containment of living cells within a polymeric matrix or membrane. The purpose of this review is to describe the various bioencapsulation techniques developed to encapsulate and thus immunoisolate pancreatic islets or β-cells toward the engineering of a biomimetic endocrine

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pancreas. This will be approached initially by a discussion of the various tissues and cell lines of interest, current pancreas and islet implants, encapsulation technologies for islet immunoisolation, and the properties of alginate, the most commonly implemented encapsulation material. New and novel encapsulation approaches will also be described.

2. Pancreas anatomy and transplantation

The pancreas is predominantly composed of exocrine cells which produce digestive enzymes and buffers for excretion into the duodenum *via* the pancreatic duct. Only about 1% of the pancreas is occupied by endocrine structures known as islets of Langerhans, which are scattered throughout the pancreas and account for roughly two million cells in the average adult human. Islets are heavily vascularized *via* a capillary network supplied by the pancreaticoduodenal and pancreatic arteries, and drained by the hepatic portal vein, which flows directly into the liver [1].

Islets are composed primarily of five endocrine cell types. α-Cells produce glucagon, which raises blood glucose by increasing the rate of glycogen breakdown and hepatic glucose release. β-Cells produce insulin, which lowers blood glucose by increasing the rate of glucose uptake in most cell types. Composing roughly 85% of the cells within an islet, β-cells are the most prevalent [2]. δ-Cells produce somatostatin, inhibiting production and secretion of both glucagon and insulin while slowing the rate of food absorption and enzyme secretion in the digestive tract. F-cells (pp-cells) produce pancreatic polypeptide, which inhibits gallbladder contractions and regulates the production of some pancreatic enzymes. Finally, ε-cells produce the hormone ghrelin to stimulate hunger [2]. Only α and β-cells are sensitive to blood glucose levels, and are therefore the most prominently discussed and observed cells for the purpose of endocrine pancreas engineering [3].

The insulin precursor proinsulin is synthesized by β-cells, which then cleave it into the 51 amino acid, 5808 Da insulin molecule composed of A and B chains joined by disulphide bonds [4]. The byproduct of insulin production, cleaved C-peptide, is often quantified to measure the rate of insulin production. As C-peptide differs between mammalian species, blood levels can be monitored during *in vivo* studies to determine if insulin is being produced by a xenogenic transplant or by residual host pancreatic tissue [5].

Type I diabetes mellitus is a disease resulting from the destruction of pancreatic islets, most importantly the insulin-producing β-cells. When insulin production is absent or insufficient, the concentration of glucose in the blood accumulates, a condition termed hyperglycemia. Chronic hyperglycemia results in serious medical complications including atherosclerosis, kidney failure, blindness, peripheral nerve damage, stroke, coronary heart disease, limb amputation and depression [6,7]. Obversely, low blood sugar, termed hypoglycemia, is also a serious concern for those with diabetes, as unregulated blood glucose levels are highly labile and can fall to low levels with little warning, resulting in unconsciousness, brain damage, and finally death if unresolved [8].

Currently, Type I diabetes is routinely managed by multiple daily insulin injections, blood sugar monitoring, and carefully controlled diet and exercise. However, these non-continuous monitoring and adjustment schemes simply retard the progression of the disease [9]. In addition, insulin therapy to reduce hyperglycemic spikes decreases the risk of complications but increases the risk of life-threatening hypoglycemic episodes [10].

Type I diabetes only accounts for 10% of patients with diabetes, however it develops much earlier, traditionally during adolescence, resulting in an increased number of chronic complications and a 15 year average reduction in life expectancy [11].

The increasing cost of diabetes treatment and the resulting secondary effects have promoted continued effort and funding to combat diabetes and the detrimental effects of labile blood sugar, in a manner which is more controlled and biomimetic. This review will focus on cell-encapsulation based therapies for Type I diabetes, which are an important subset of the diverse and exciting fields of biological and pharmacological therapies for the treatment of diabetes.

2.1. Whole pancreas transplant

When pharmacological alternatives are insufficient, the most direct approach to replacement of endocrine tissue in Type I diabetes is whole pancreas transplantation. Xenogenic pancreas transplantation in part or as a minced injectable, was used as early as 1894, almost 30 years before the discovery of insulin, for the temporary relief of the symptoms of diabetes mellitus, which at the time was inevitably fatal [12]. Allogenic whole pancreas transplantation was first performed in 1966, and since then over 35,000 transplants have been reported to the International Pancreas Transplant Registry [13]. However, unlike heart, lung, and liver transplants, whole pancreas transplantation is not a life-saving operation, but one to improve quality of life. The long-term advantages of glycemic control must be balanced against the severity of the procedure and the side-effects of life-long immunosuppression which is currently required to prevent alloimmunity and autoimmune reoccurrence [14]. In addition, the number of donors with transplant-quality pancreases is very low [15].

Therefore, whole pancreas transplant can only be justified for a small subset of patients and another approach is needed to improve glycemic control in the majority of the population afflicted with Type-1 diabetes. The first step in the engineering of a cell-based treatment for diabetes is to discard the bulk of the pancreas, the superfluous exocrine tissue, and focus on the endocrine islets of Langerhans.

2.2. Islet transplant

Implantation of whole islets, initially proposed by Lacy and Kostianovsky [16], is preferred over whole-pancreas or purified β-cell transplantation, as islets can be easily isolated, quantified, and implanted, while retaining the complex multicellular interactions of the glucose-modulating endocrine pancreas functional subunit [2]. Dividing islets further into their constituent cells and implanting purified insulin-producing β-cells reduces the body's ability to suppress insulin secretion during a hypoglycemic episode. Islet dissociation also eliminates the cell–cell interactions found within the aggregated islet state required for glucose responsiveness and the up-regulation of insulin secretion [17].

Islet implantation sites are typically located such that secreted insulin enters portal venous circulation, either through the hepatic portal vein or through venous drainage of the peritoneal cavity. Hepatic drainage then replicates natural hormone delivery from the pancreas to the hepatic portal vein *via* the pancreatic, pancreaticoduodenal and splenic veins [1]. It is noteworthy that when islets are transplanted into the liver, the insulin is drained into the sus-hepatic circulation and reaches the hepatic cells only through the general circulation.

2.3. Islet isolation

Human islet isolation for transplantation, made famous by the Edmonton Protocol [18] requires the proper collection, digestion, and purification of the pancreas. Freshly procured pancreases are held in chilled organ transplant solution such as ViaSpan®. The pancreatic duct is then infused with chilled protease solution, generally a mixture of collagenases and thermolysin such as Liberase [19], to digest the pancreatic extracellular matrix (ECM). Islets are then separated from the ECM by gentle mechanical dissociation and purified *via* a density gradient of Ficoll–diatrizoic acid in an apheresis system. In place of xenoprotein products, the isolation and purification media for the Edmonton Protocol contains 25% human albumin [18]. In 46% of clinical applications, islets are used without pretreatment, and in the remaining 54% of cases, islets are cultured for 27 h on average, allowing the islet

population to be checked for contamination while maintaining viability and providing time to prepare for transplantation [20].

Table 1 summarizes islet yields from human-to-human transplants reported to the CITR between 1999 and 2008. The Islet Equivalent (IEQ) is a standard volumetric unit of measure for islet studies, defined as a sphere with a diameter of 150 μm. It is important to take notice of the number of IEQ isolated from a single adult pancreas, as many techniques use cell quantities far higher than the physiological quantity, requiring multiple donors [21].

2.4. Hepatic infusion

Many different implantation techniques have been developed for both human and animal models. The most widely instituted islet transplantation method in human-to-human clinical applications is intraportal hepatic infusion [20]. The first case of human insulin-independence after hepatic infusion of pancreatic islets was reported in 1989 [22]. The Collaborative Islet Transplant Registry reported that between 1999 and 2009, 1072 human-to-human islet transplant procedures were performed on 571 patients at 41 transplant centers in North America, Europe, and Australia [23]. For those wishing to learn more about clinical islet transplantation, the CITR annual report is a wealth of in-depth statistical information.

The most prominent breakthrough in the field of islet transplant occurred in 2000 when the Edmonton Protocol by Shapiro et al. [18] resulted in seven out of seven islet recipients becoming insulin-independent at one year, with steroid-free immunosuppression. Islets were infused into the patient's main portal vein over 2–3 separate procedures requiring a total of 11,500 ± 1600 IEQ/kg to achieve insulin independence, compared to the 4620 ± 1920 IEQ/kg found in the average donor. No patients experienced acute rejection.

The restoration of normoglycemia with glucocorticoid-free immunosuppression was a sign that the field was maturing into a clinically viable treatment for Type I diabetes. However, an international trial of the Edmonton Protocol in 2006 [24] was less successful at achieving insulin independence. The study involved 36 patients at 9 sites, only 16 of whom (44%) achieved insulin independence at one year. Of the remainder, 10 had partial function and 10 had complete graft loss. The poor results were attributed to inexperience with the procedure. At sites where four or more procedures were performed in the preceding two years, 12 of 18 patients (67%) achieved insulin independence at one year, while only 4 of 18 (22%) patients at less experienced sites achieved the same result.

Although the international clinical trial failed to match the success rate of the initial study, it succeeded in standardizing human pancreas selection, islet processing, product-release criteria, recipient selection, and post-transplantation care under a FDA investigational new drug submission, while highlighting the need for less toxic immunosuppressive therapy [24].

Of the 279 hepatic islet-transplant patients registered by the CITR between 1999 and 2004, 23% retained insulin independence at 3 years post first infusion, 29% maintained detectable levels of c-peptide, despite resuming exogenous insulin therapy, 26% had complete graft failure with no detectable c-peptide levels, and 22% had unreported outcomes at 3 years [25]. Despite low levels of insulin independence, many patients report reduced exogenous insulin demands and a significant reduction in hypoglycemic episodes.

While intraportal infusion provides ready access to systemic circulation, it also exposes the islets to high concentrations of toxic immunosuppressive drugs [26]. It has also been shown to increase the risks of thrombogenesis by the blood-mediated inflammatory reaction to the implanted islets, resulting in the immediate loss of half the grafted islet mass [27]. For these reasons, as well as the inability to retrieve the infused islets from the liver following infusion therapy, other implantation sites and methods have been investigated.

Table 1
Summary of human-islet isolation data from human-to-human clinical trials (modified from exhibit 3–4A in CITR 2009 report [20]; used with kind permission of CITR).

	N	Mean	SD	Min	Max
IEQ post purification ($\times 10^{-3}$)	403	384	156	85	973
Total cell volume (mL)	805	3.5	2.1	0.1	16.0
Islet particle count ($\times 10^{-3}$)	683	364	158	63	996
IEQ ($\times 10^{-3}$)	721	388	162	54	1122
IEQ ($\times 10^{-3}$)/kg donor weight	700	4.62	1.92	0.72	17
Beta cells ($\times 10^{-6}$)	266	264	179	4	975

2.5. Subcutaneous implantation

The subcutaneous space provides a site for islet implantation which is much easier to monitor and revise in the event of an excessive immune response or future advancements in the field, relative to hepatic infusion, however the subcutaneous space is also poorly vascularized [3,28]. To overcome this limitation, studies performed on the prevascularization and concurrent vascularization of the subcutaneous space are widely published [3,29,30].

Hiscox et al. [29] used a system of prevascularized collagen gels to enhance islet cell survival in subcutaneous studies by maintaining the intraislet endothelial cell structures. Islets placed within the prevascularized structures maintained the ability to produce insulin longer than controls and produced more insulin with fewer IEQ, while remaining responsive to external stimuli. Perez et al. [3] investigated the subcutaneous implantation of islets within plasma–fibroblast gel scaffolds. The use of such a readily biodegradable material provides an initial post-transplant support matrix while enabling the development of a capillary bed and promoting the recovery of extracellular matrix (ECM) interactions through natural tissue remodeling [31]. Culture in 3D fibrin gels has also been shown to improve *in vivo* islet function, survival and differentiation [32–34].

The plasma–fibroblast gel system of Perez et al. [3] has only been implanted in an immunocompromised nude mouse model to date, but shows the promise of protein-based scaffold designs. Their gel incorporated 3000 IEQ and 6.0–7.5 × 10^4 fibroblasts in a 4 mL plasma gel, all components isolated from Wistar rats. Upon implantation into STZ-induced diabetic nude mice, normoglycemia was restored at 3.9 ± 1.2 days and was maintained for the 60 day test period in all seven animals. Histological evaluation of the implant after 60 days showed full incorporation into the subcutaneous space, complete with vasculature and collagen matrix.

2.6. Islet immunoisolation

While studies on immunosuppressed patients and animal models, including the hepatic infusions of Shapiro et al. [18,24,26] and the subcutaneous transplantations of Perez et al. [3] are promising, these systems will need to be modified to work in a non-immunosuppressed allogenic model before they can be broadly implemented in the clinic. Current systemic immunosuppression therapies for the treatment of diabetes by transplantation are only warranted for patients who have exhausted all other glycemic control strategies, due to the serious repercussions of chronic immunosuppression [24]. Beyond endangering the health of the patient, islet-transplant immunosuppression regimes may also reduce the effectiveness of the therapy, as they have also been shown to reduce the viability and glucose-responsiveness of rat and pig islets *in vitro* [35].

The history of graft rejection and toxicity of systemic immunosuppression have resulted in extensive investigation of immunoisolation to enhance the efficacy of transplant techniques. Microencapsulation, the most commonly studied immunoisolation technique, has a proven ability to protect cells from the main host immune response [36–38]. The elimination of systemic immunosuppression therapy enables the

use of a tissue-engineered endocrine pancreas approach in a much larger group of patients.

When initially proposed, the goal of immunoisolation was to use microencapsulation membranes with a low molecular weight cut-off to allow the diffusion of nutrients, wastes and hormones, while preventing all contact between graft tissue and the host immune system. However, studies into alginate membranes with molecular weight cut-offs larger than immunoglobulin G (~150 kDa) have proven successful, indicating that blocking antibodies and cytokines may not be a requirement of immunosuppression [39]. It is believed that the main purpose of the semi-permeable membrane is to act as a physical barrier, preventing antigen recognition by the host T cells. However, studies continue to show that reduced molecular-weight cutoffs in capsules, specifically in the 150 kDa range, are correlated with decreased fibrotic encapsulation and anti-graft host immune response *in vivo* [40]. Solutions to low-molecular weight antigen shedding from within the capsules and interleukin-1β diffusion into capsules continue to be investigated [41,42].

3. Microencapsulation

Thomas Chang introduced the use of semipermeable microcapsules for the encapsulation of enzymes in 1964, with a brief mention of the potential for cellular encapsulation [43]. The publication recognized as the primary inspiration of modern bioencapsulation research was that by Lim and Sun in 1980 [36] for their study of encapsulated pancreatic islets within calcium alginate microcapsules.

Microencapsulation within spherical microparticles possesses many benefits over other encapsulation geometries, including a high surface area to volume ratio, a high resistance to mechanical stress, a relatively short diffusion path length, and access to a number of implantation sites by injection [44]. Immunoisolation in microcapsules has an additional benefit in that it retains any apoptotic or necrotic cells and cell debris within the capsule. In a preliminary *in vitro* study, these cells would otherwise be shed from the islet and be removed by medium changes, skewing viability analyses. For *in vivo* and clinical applications, the same intrinsic retention of dead and necrotic cells greatly reduces or eliminates the immune-response associated with the release of allogenic and xenogenic cellular debris [45].

The most widely implemented microcapsule for islet immunoisolation is the ionically crosslinked alginate-poly-amino-acid-alginate (APA) system [46]. Islets are entrapped within alginate beads produced through a variety of methods, most often by assisted dropwise extrusion of islets suspended within an alginate solution into an aqueous calcium chloride gelation bath as illustrated in Fig. 1. Ionically crosslinked alginate capsules entrapping islets are then added to a solution of cationic poly-amino acid, commonly poly(L-lysine) (PLL) or poly(L-ornithine) (PLO) which forms a well-defined semi-permeable membrane around the polyanionic alginate. The final step is to re-incubate in a dilute alginate solution, forming an additional ionically associated alginate layer, to reduce the immunogenicity of the cationic poly-amino acid membrane [2].

Barium chloride may be used to replace calcium chloride, producing stronger capsules with less susceptibility to dissolution in growth medium over extended periods of culture [47].

Passive diffusion of nutrients and wastes within alginate microparticles necessitates short diffusion pathlengths. The rule of thumb is that no islet should be more than 200 μm from the surface of the particle as that is the maximum distance for effective oxygen and nutrient diffusion in most tissues [48]. Some groups have added an additional safety factor, such that the average capsule should be no larger than 300 μm, allowing for variations about the mean while staying safely within the maximum 200 μm pathlength for even the most central cells of the islet. This fine size necessitates a refined particle production technique.

Simple dropwise extrusion from a needle into a bath of divalent ions (Ca^{2+} or Ba^{2+}) relies on gravity to overcome the surface tension of the droplet at the tip of the needle, producing particles with diameters approaching 2000 μm [49]. Detachment of the drop from the needle tip occurs when the droplet mass exceeds the surface tension forces:

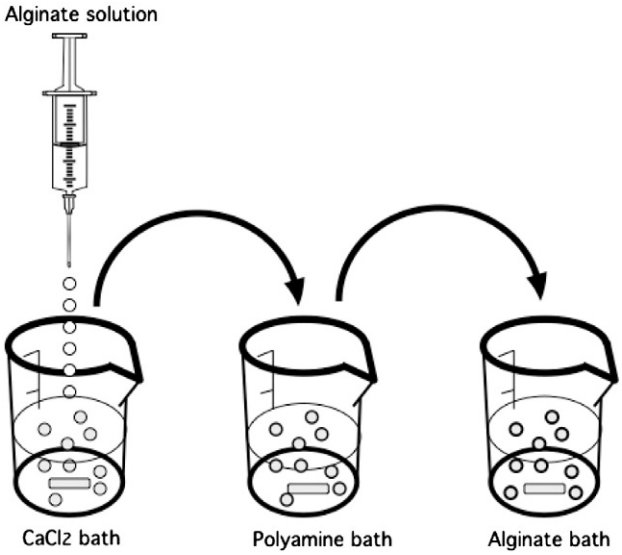

Fig. 1. Formulation of alginate/poly-amino acid/alginate (APA) microcapsules.

$$d = \sqrt[3]{\frac{6d_e\gamma}{g\rho}}$$

where d and d_e are the droplet and needle diameters, γ is the surface tension and ρ is the liquid density. The reduction in droplet diameter is a function of the cube root of external needle diameter, surface tension, and solution density. Therefore, when optimizing particle diameters from an aqueous solution of alginate with relatively fixed density and surface tension, the practicality of reducing the external needle diameter is limited, and another approach must be investigated.

3.1. Electrostatic encapsulation

Electrostatic-mediated droplet production is effective, cytocompatible, and introduces no additional sources of contamination. In this technique, islets are suspended in a dilute (1–4% w/v) alginate solution which is dispensed through a needle charged with ~11 kV of potential generated by a high voltage power source as illustrated in Fig. 2. The divalent cation collection bath is grounded and placed far enough from the tip of the needle to prevent arcing, but close enough to produce a stable electrical field to guide the droplets toward it. The difference in potential causes an accumulation of charge on the surface of the droplet which reduces surface tension by a factor of $(1 - U^2/U_{cr}^2)$ where U is the potential applied, U_{cr} is the critical potential required to produce a jet of solution, and $U < U_{cr}$ [49,50]. Pulsing the potential at frequencies of ~40 Hz in a square wave pattern breaks the flow into discrete droplets of uniform size, smaller than those achieved without electrostatic assistance. While there are many benefits, including cytocompatibility, sterility, and simplicity, one issue with the technique is limited scale-up potential. Flow rates for 300 μm diameter particles are on the order of 5 mL/h, thus the approach to scaling the process typically involves additional extrusion needles and larger collection baths.

3.2. Vibrating nozzle encapsulation

One approach used to scale up bead production is to increase the liquid extrusion rate forming a liquid jet at the tip of the needle. Breakage

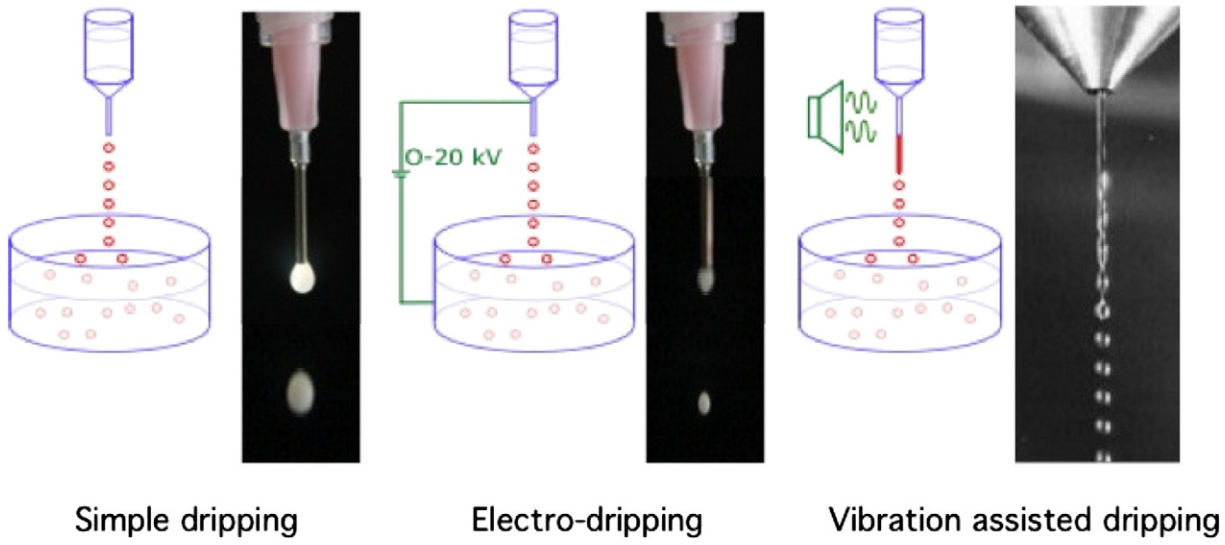

Fig. 2. Droplet extrusion technologies from simple dripping to electro or vibration assisted methods.

of the jet into discrete droplets or beads is possible through vibration [51] as shown in Fig. 2, or using a jet cutting tool [52].

Liquid jets will naturally break into droplets at a particular frequency, *f*:

$$f = \frac{u_j}{\lambda}$$

and

$$\lambda = 4.058\ d_j$$

where u_j and d_j are the linear velocity and diameter of the jet respectively, and λ is the vibration wavelength [53]. The jet will be in resonance at this frequency, resulting in jet breakup into nearly uniform diameter with a diameter which can be approximated to about double the internal diameter of the needle. An illustration of a jet breakup device is illustrated in Fig. 3.

Increased productivity of small diameter beads is the main advantage of this technology, while the main disadvantage is that liquid jets generally do not break for liquids with viscosities greater than 200 mPa.s, due to vibration damping. One approach for higher viscosity fluids is to use a jet cutter consisting of a rotating wheel equipped with fine wires [52]. The problem in this case is that high shear forces can damage large cells and cell clusters, and there will be difficulties maintaining aseptic conditions.

3.3. Emulsion based encapsulation

The challenge of limited scale-up potential has been addressed by the emulsification with the triggered internal gelation technique. This novel system, developed by Poncelet et al. [54] separates particle formation and ionic gelation. Alginate droplets formed in an oil/water emulsion are ionically crosslinked by the pH-triggered release of Ca^{2+} from an insoluble $CaCO_3$ complex within the emulsified droplet. Discrete ionically-gelled alginate microparticles are formed virtually instantaneously with no limitations on batch size, as long as adequate mixing is available to sustain a stable emulsion [54,55]. Like the dropwise methods, particles are formed from a dilute solution of sodium alginate, however in the emulsion system it is supplemented with ultra-fine insoluble calcium carbonate. One part of the alginate aqueous phase is added to three parts oil and emulsified. The emulsion is allowed to stabilize for 15 min before the pH is decreased by the addition of acetic acid dissolved in an aliquot of the oil phase. At pH 6.5, calcium is solubilized, ionically crosslinking the alginate, forming spherical microparticles.

One issue with the standard emulsion system is a wide particle size range [54,56]. To ensure optimal diffusion kinetics and complete islet encapsulation, a narrower size range is preferred [57]. This has been partially solved through the application of static mixers in place of mechanical impellers to produce the emulsion as illustrated in Fig. 4. The resulting particles have a significantly narrower size distribution.

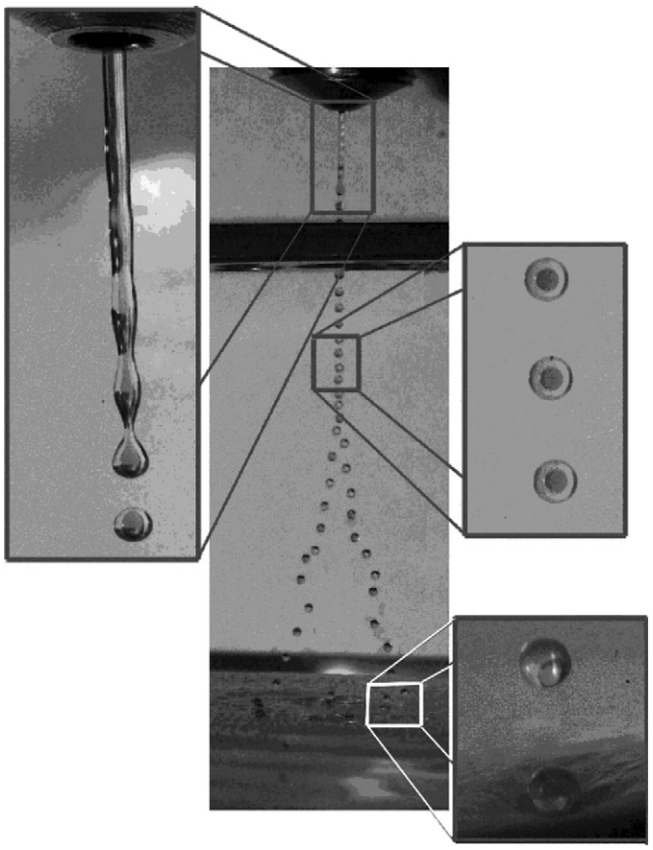

Fig. 3. Jet breakage by resonance (Inotech).

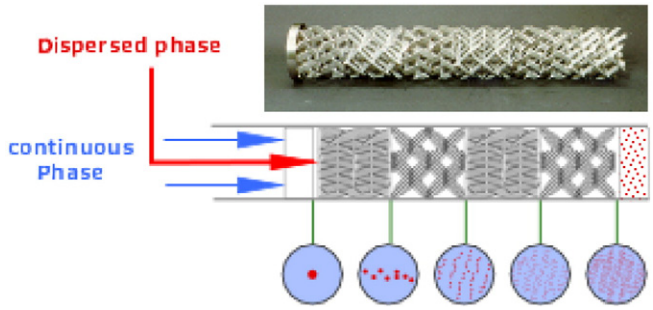

Fig. 4. Encapsulation *via* emulsion production using a static mixer.

The density distribution of the alginates in internally-gelled capsules formed by emulsion gelation is different from that of externally gelled particles formed by droplet extrusion. Internally gelled particles are more homogenous as the calcium is liberated throughout the particle upon acidification. Externally gelled particles rely on the diffusion of calcium or barium into the particle, resulting in a denser periphery able to withstand high shear forces [58]. There are advantages and disadvantages of inhomogeneous alginates compared to homogenous alginates. For example, a higher alginate density at the bead periphery may have the advantages of reducing the molecular weight cut-off and to decrease islet bulging at the capsule surface.

The internal gelation emulsion system developed by Poncelet et al [54] was optimized for mammalian cells by Hoesli et al. [56] as illustrated in Fig. 5. Using buffered 1.5% alginate solutions and a protocol with accelerated emulsion and acidification steps, they achieved a post-encapsulation islet viability of 90 ± 2% for MIN6 and 71 ± 4% for primary pancreatic tissue within particles 757 ± 20 μm in diameter. Survival rates were on-par with electrostatic particle formation.

In a subsequent investigation, Hoesli et al. [40] further optimized the internal gelation emulsion technique with 5 w/v% alginate beads encapsulating β-TC3 cells, a murine insulinoma cell line. The high alginate concentration, impractical for extrusion, was assessed relative to conventional 1.5% alginate beads produced by external gelation. A study in STZ-induced diabetic mice exhibited a significant blood glucose reduction after 2 days relative to 19 days for the 1.5% alginate beads. The 5% emulsion beads had a reduced molecular weight cutoff profile relative to the control, no measurable graft-reactive IgG at 20 days *in vivo*, and no fibrotic overgrowth at 56 days compared to the 1.5% extruded control with 32% overgrowth.

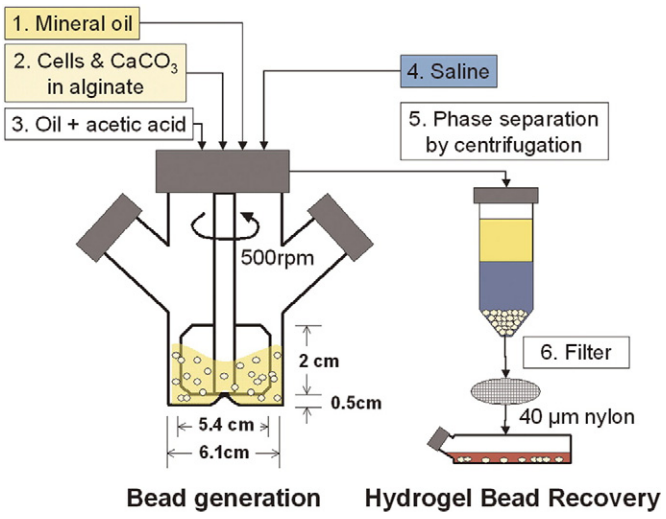

Fig. 5. Emulsion encapsulation system optimized by Hoesli et al. for mammalian cell encapsulation (Fig. 1 from [56]; used with permission, Copyright © 2010 Wiley Periodicals, Inc.).

The increase in performance from the 5% calcium alginate emulsion particles produced by internal gelation, with the β-TC3 cell-line, relative to traditional external gelation shows this technique has merits not only in ease-of-scale but also in superior performance relative to the standard technique.

Hoesli et al. [40] contributed significantly to encapsulation techniques for the therapeutic treatment of diabetes. However, their system remains a bio-inert alginate capsule which isolates but does not promote the performance of the encapsulated cells.

3.4. ECM-based fibrous scaffolds

Alginate, which is still the best material to form the core bead of the microcapsule, is a polysaccharide and as such has no domain for the attachment of integrins. Integrins are involved in cellular adhesion but also in the transmission of signals that are important in cell survival, differentiation and multiplication. Therefore, developing methods to incorporate ECM-based molecules within alginate beads is of critical importance for the long-term survival of encapsulated cells.

The peri-islet microenvironment is far from inert, consisting of collagens I–VI, laminin, fibronectin, elastin, nidogen/entactin, vitronectin, heparin sulfate, and chondroitin sulfate [59]. The influence of these ECM-based factors acting *via* integrin and non-integrin mediated interactions has been proven to increase β-cell survival, decrease apoptosis, maintain islet morphology, and increase insulin production in response to glucose stimulation [3,59–61].

While Del Guerra et al. [62] incorporated protein-based porous matrices within immunoisolated alginate particles, the process dissociated the islets, eliminating the cell–cell interactions found within the aggregated islet state and reducing insulin secretion relative to intact islet controls [17,62]. However, the presence of the porous gelatin matrix did significantly increase glucose-induced insulin production relative to dissociated controls [62]. Therefore, matrices, while beneficial, must be investigated on a scale that allows the seeding of intact islets, which average of 150 μm in diameter. To seed a preformed porous matrix, the pores must be 5–10 times the size of the islets to ensure homogenous seeding [63], thus rendering traditional porous matrices too large for immunoisolation within 300 μm particles.

Cultures of whole islets on matrices of synthetic microfibers have also shown increased viability and insulin secretion [64–68]. The addition of basement membrane proteins to the surface of these scaffolds, including collagen I, IV, laminin, and fibronectin have been proven to further increase islet viability and insulin secretion [66,69–72]. The use of ECM-based components, as simple as collagen I, and structural fibrous scaffolds have been shown to work synergistically to increase insulin production and gene expression in islets [72].

Steele et al. [73] were the first to combine the physical benefits of a microfibrous scaffold and chemical cues of ECM-derived components within an immunoisolated alginate particle for the culture of intact pancreatic islets. The design utilized crosslinked gelatin microfibers to form a matrix around isolated rat islets, incorporated into a traditional one-step alginate encapsulation process using electro-static mediated droplet extrusion. The production of discrete, crosslinked gelatin fibers ~20 μm in diameter was achieved by a vortex–extrusion method developed for the encapsulation technique. Particles composed of 1.6% (w/v) barium-alginate containing 40% (v/v) gelatin fibers (Fig. 6) were used to encapsulate primary rat islets. Islet viability was significantly improved relative to fiber-free controls over 21 days *in vitro*. A distinct change in islet morphology was also observed in the particles, with the fiber-free control group losing their aggregated morphology, breaking apart into dissociated live β-cells at a rate of 31% of islets by day 21, *versus* 8% for the fiber-scaffold group (Fig. 7A). Upon mechanical compression of the alginate particles, distention of the islet aggregates was observed

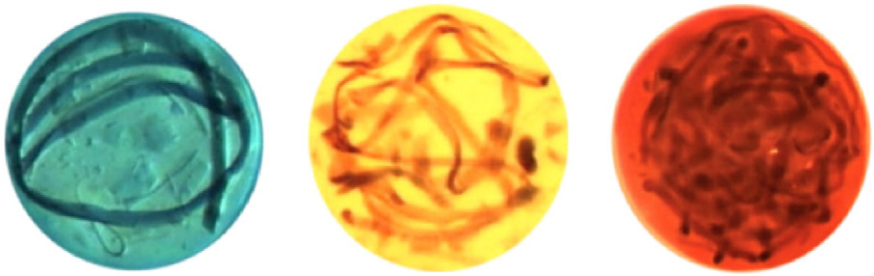

Fig. 6. Calcium alginate microcapsules approximately 300 μm in diameter, loaded with gelatin microfibers at various concentrations [73] (used with permission, Copyright © 2012 Wiley Periodicals, Inc.).

due to the adhesion between the outer cells of the islet aggregate and the gelatin fibers (Fig. 7B).

This technique combines the benefits of a fibrous matrix support, ECM-based biomolecule recognition and alginate encapsulation. The study by Steele et al. [73] confirms significant benefits from this simple yet effective alteration to the traditional encapsulation system.

4. Alginate

Considering that alginate is the primary polymer used for islet encapsulation, it deserves some discussion in terms of its properties and purification protocols. Alginates are polysaccharides composed of unbranched 1–4 linked α-L-guluronic acid (G) and β-D-mannuronic acid (M) (Fig. 8), derived primarily from the brown algae *Laminaria hyperborean*, *Macrocystis pyrifera*, and *Ascophyllum nodosum* [74]. The organization of the G and M monomers within the polymer is highly variable with blocks of G and M interspersed with alternating MG sequences [74]. Divalent cations, such as Ca^{2+} and Ba^{2+} bind to G blocks on neighboring chains, forming junction zones, leading to an ionic gel. High-G content alginates produce capsules with high mechanical strength, while high-M alginates exhibit elastic properties [38].

As alginate gels are stabilized by divalent cations, capsule mechanical integrity is sensitive to the presence of chelating agents including EDTA, citrate, phosphate and lactate, as well as monovalent cations such as Na^{+} [74].

4.1. Alginate purification

The largest issues with alginate-based microcapsule systems to date have been standardization, stability and biocompatibility [75,76]. High-quality alginates are produced from algal stipes collected directly from the sea, peeled and treated with antimicrobial agents to reduce the cytotoxic gram-positive debris. Lower-grade alginates are produced from algae which washed ashore and have significantly higher contamination levels that are difficult to remove from the processed product. In both cases, alginate is extracted from the algae by EDTA chelation, physical sieving, precipitation with ethanol and KCl and finally lyophilization [38].

Mallet and Korbutt [75] investigated the effect of further purification of pharmaceutical-grade alginate on encapsulated murine islet survival in the peritoneal cavity of STZ-induced diabetic mice for a period of 105 days. The islets were encapsulated by electrostatic-assisted dropwise extrusion of 1.5% alginate solution containing 10,000 IEQ/mL into a $CaCl_2$ collection bath. The purified alginate particles had an islet survival rate of 90.5% relative to 69.2% for a commercial alginate, with dramatically reduced capsular overgrowth and superior insulin secretion.

More extensive alginate purification protocols, such as the Klöch method, have been developed and further optimized to reduce cytotoxic protein levels [77]. Ménard et al. [78] verified the efficacy of the modified Klöch method of alginate purification which entails chloroform extraction, activated and neutral carbon treatment, sterile filtration, acetic acid wash, sodium citrate wash, ethanol extraction, saline dialysis, precipitation and lyophilization. The entire process required 11 days and significant expense to purify a pharmaceutical-grade starting product. However, the benefits of the modified Klöch method are substantial. Langlois et al. [77] encapsulated rat islets in purified alginate microparticles and observed significant improvement in insulin secretion and an increase in viability at 14 days from 18% to 48%.

5. Other immunoisolation techniques

In addition to microencapsulation in alginates, a number of other techniques have been proposed for the immunoisolation of islets. The

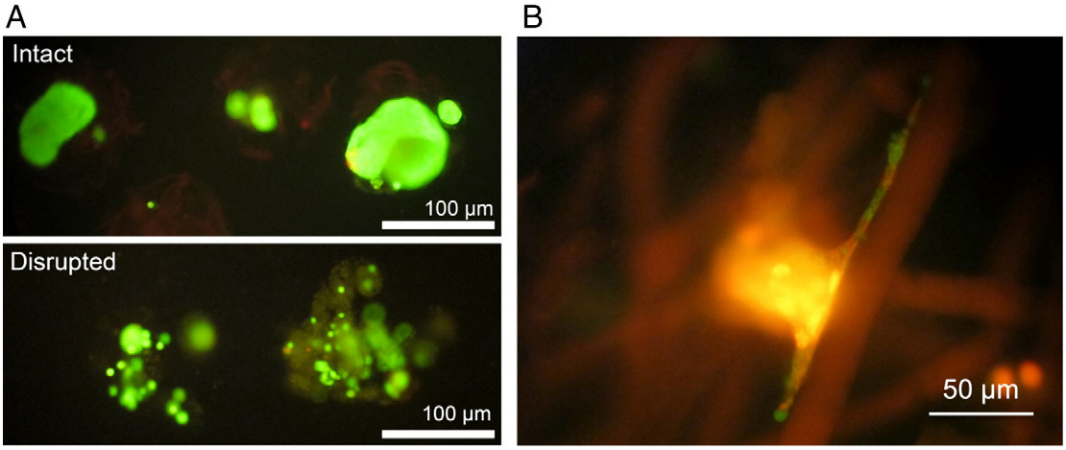

Fig. 7. A: Example of intact *versus* disrupted islet morphology, visualized with acridine orange viability stain (green) and auto-fluorescent genipin-crosslinked gelatin microfibers (red) present in the intact image only. Both intact and disrupted images depict alginate-encapsulated islets at day 21. B) Viable cells (green) from an encapsulated islet adhered to a gelatin fiber. [73] (used with permission, Copyright © 2012 Wiley Periodicals, Inc.).

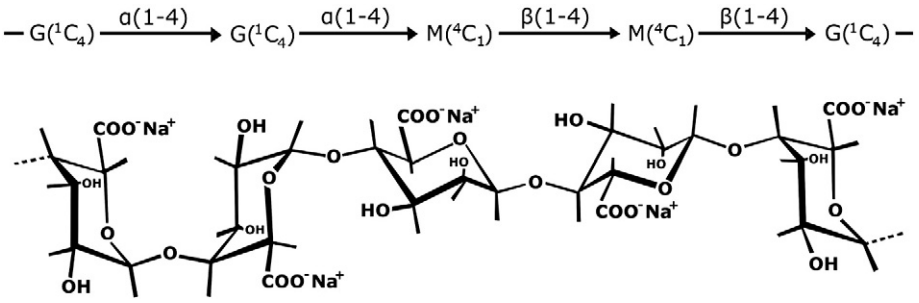

Fig. 8. Chemical structure of sodium alginate, composed of guluronic acid (G) and mannuronic acid (M) monomeric units (modeled after [74], Fig. 2).

wide range of techniques can be roughly divided into intravascular and extravascular devices.

5.1. Intravascular immunoisolation devices

Intravascular devices were of considerable interest in early islet studies as they solved the problem of ischemic conditions outside the vasculature by placing islets within semi-permeable membranes in contact with the systemic circulation. Many devices, such as that used by Monaco et al. [79] were synthetic devices grafted directly to the vasculature. While proving successful in large animal models, including the induction of normoglycemia in dogs for up to a year, the constant risk of thrombosis required severe systemic anticoagulation regimens. Despite the anticoagulation measures taken, thrombosis continued to be an issue, along with complications associated with vascular prosthetic surgery, and infection [80]. In addition, the polymer (PTFE) used to construct the devices was shown to induce the production of interleukin-1 beta (IL-1β), a cytokine lethal to islets [81]. Intravascular devices also require more invasive procedures with higher risks of both short term and long term complications.

The possibility of infusing the hepatic portal vein with encapsulated islets in reduced capsule size has been investigated. Ten thousand particles smaller than 315 μm can be infused with only a short and moderate increase in portal vein pressure immediately following injection, returning to normal levels within two hours [82]. In comparison, the same number of particles at 420 μm in diameter caused a considerable increase in portal vein pressure, resulting in death within 3 h for 2 of the 3 rats.

5.2. Extravascular immunoisolation devices

Due to the risks associated with vascular anastomosis, recent work in immunoisolation has been focused on extravascular approaches, focusing primarily on microcapsules. However, macrocapsules have also been investigated.

Macrocapsules containing large numbers of islets have the advantage of easy retrievability and minimally invasive implantation. The production of macrocapsules from hydrogels has increased biocompatibility by reducing interfacial tension, protein absorption, and cell adhesion, relative to the older system of poly(acrylonitrile) and poly(vinyl chloride) diffusion chambers [83]. Muthyala et al. [84] encapsulated mouse islets grown on a crosslinked gelatin matrix within a poly(urethane)-poly (vinyl pyrrolidone) macrocapsule 18 ± 7 mm in length, 4.0 ± 0.5 mm in diameter and 1.0 ± 0.2 mm in thickness. An *in vivo* study in rats achieved euglycemia after 15 days, which was sustained for up to 90 days. In the absence of the gelatin scaffold, islet death was observed within 45 days. Macrocapsules suffer from nutritional limitations, necessitating a cell volume fraction of <10% to guarantee adequate feeding [83].

Fibrous systems incorporate many of the benefits discussed previously, including the inclusion of ECM proteins and low diffusional distances. Townsend-Nicholson and Jayasinghe (2006) were the first to encapsulate living cells in electrospun fibers, using a core–shell method, encasing a suspension of immortalized cells in culture medium within an outer shell of PDMS [85]. More recently, the encapsulation of living cells within core–shell microfibers has been developed in a highly-controlled microfluidic device, capable of producing meter-long continuous fibers with full cell–cell contact [86]. Fibers loaded with dissociated rat islets suspended within a collagen I gel, immune-isolated in an outer core of alginate–agarose interpenetrating network hydrogel, and injected beneath the kidney capsule of a diabetic mouse were able to maintain normoglycemia for 2 weeks. Due to the microfluidic production method, the fibers can be woven, wound and braided with other fibers to produce robust, removable multi-cell systems. The cell–cell contact afforded in this system may be sufficient to reduce the impact of islet-dissociation on long-term glucose modulation.

6. Human trials with immunoisolating microcapsules

Neonatal porcine islets were encapsulated in alginate-PLL-alginate capsules and transplanted in 1996 into the peritoneal cavity of a Type I diabetic man at 15,000 IEQ/kg without immunosuppression [4]. The patient was able to reduce exogenous insulin by up to 30% at 12 weeks, only returning to pre-transplant levels at week 49. A follow-up study was conducted 9.5 years later to assess the long-term effects of the treatment [4]. *Via* laparoscopy, nodules were observed throughout the peritoneum and sampled. The capsules contained live islets which stained for insulin and glucagon. The capsules were unchanged in size, but had become opaque and rigid. The patient claimed to have better glycemic control at 9.5 years, compared to pre-transplant, with a reduction in hyperglycemic episodes and better overnight blood sugar control. The primary conclusion of the follow-up study was that xenogenic islets immunoisolated in alginate-PLL-alginate beads implanted without immunosuppression did not lead to any detectable immune response or fibrosis and achieved partial graft survival for a period of 9.5 years.

With proven success in animal models, immunosuppressed, and xenogenic immunoisolated human trials, clinical trials of allogenic immunoisolated islets were launched. Calafiore et al. [87,88] combined the islet extraction techniques from the Edmonton Protocol with the alginate-PLO-alginate immunoisolation system to conduct non-immunosuppressed human clinical trials, setting standard operative procedures for human islet encapsulation and transplantation. In the first two human trials of the Calafiore protocol [87], islets were isolated from donor pancreases, cultured for 24 h, and encapsulated. The beads were formed by the extrusion of endotoxin and pyrogen-free sodium alginate (1.6%) into a $CaCl_2$ bath, with an average bead diameter of 500 μm and 1–2 islets/bead. Coatings were formed by incubation in 0.12% and 0.06% PLO and finally 0.04% sodium alginate. The coated beads were injected into the peritoneal cavity under local anesthetic in 150 mL of saline solution through a 16 gauge needle (400,000 and 600,000 IEQ for patients 1 and 2, respectively). Published results indicate that the patients were unable to eliminate exogenous insulin therapy, but had improved glycemic control at one year, with an elimination of weekly hypoglycemic episodes, and reported no adverse side effects.

However, follow-up articles on the study indicating the performance of the other 8 subjects in the 10 member study are noticeably absent.

Tuch et al. [21] outlined a four-member clinical trial for the implantation of barium-alginate encapsulated islets by intraperitoneal infusion of 340 μm beads into subjects with Type I diabetes. The clinical trial was largely unsuccessful, with no change in insulin requirements or glycemic control upon injection of approximately 180,000 IEQ on up to four separate occasions. One subject, who received four infusions, had detectable C-peptide levels for 2.5 years, but experienced no benefits from the infusion. Upon laparoscopy, mimicking a follow-up study by Elliot et al. [4], it was discovered that the capsules were intact, but contained necrotic islets completely encased in fibrous overgrowth.

The variability amongst clinical trials and between animal and human studies illuminates an issue in the field of islet microencapsulation, due partly to the lack of uniformity in material purification and characterization.

There are a number of companies currently investigating encapsulated cell-based therapies for diabetes. Living Cell Technologies is currently undertaking a 30 patient late-stage clinical trial of their DIABECELL® immunoprotected xenograft composed of alginate-encapsulated porcine islets [89]. The study is building on previous successful trials of 5000 and 10,000 IEQ/kg doses in the peritoneal cavity. While patients received important benefits including 70% reduction in unaware hypoglycemic events, and a 20% reduction in insulin dose, the requirement of exogenous insulin injection was not eliminated [90]. ViaCyte, Inc. is developing a product, VC-01™, composed of β-cell precursors derived from human pluripotent stem cells encapsulated within a retrievable, immune-protecting macrocapsule for subcutaneous implantation. The VC-01™ is currently at the pre-clinical stage with clinical testing expected within 2 years [91]. On the road to commercialization, as Novocell, they investigated PEG-encapsulated islets [92], but this has been discontinued to focus on the VC-01™ system [93]. There are two academic clinical trials of encapsulated cellular therapies for diabetes registered with the NIH. UZ Brussel is conducting a 10-patient clinical trial of alginate-encapsulated human islets implanted into the intraperitoneal cavity, under immunosuppression [94]. Finally, a team at Cliniques Universitaires Saint-Luc at Université Catholique de Louvain is subcutaneously implanting allogenic human islets into 15 patients within a 1–3 cm^2 alginate-based, immunoprotective, "monolayer cellular device" [95]. The four products summarized illustrate the wide range of products under investigation for encapsulated cellular therapy for diabetes. While the DIABECELL® clinical trials are highly promising as a therapy for Type I diabetics with highly labile blood sugar and hypoglycemic unawareness, the expense and limited lifespan of such devices will continue to be a barrier to approval by government healthcare agencies and market saturation amongst the average Type I diabetic.

7. Summary

All of the immunoisolation techniques summarized above are designed to increase the viability of the entrapped islets by protecting from threats originating outside the microenvironment, primarily the immune response. However, the long-term viability and productivity of the islets are also dependent on conditions within the system, and how well they mimic a physiological microenvironment.

The present article reviews the concepts and the progress that have been accomplished toward the development of a bio-artificial endocrine pancreas for the treatment in insulin dependent diabetes. Initially, relevant aspects of the pancreas anatomy and physiology are summarized, as well as the most important issues in the research that led to successful transplantation of pancreatic islets into immunosuppressed human diabetic patients. The concept of immune isolation of transplanted cells within semipermeable membranes or microcapsules as a method to avoid the requirement of immunosuppression is also covered. The review emphasizes methodological aspects in the production of microcapsules, using different approaches. Finally, other immune isolation techniques are summarized.

References

[1] F.H. Martini, M.J. Timmons, R.B. Tallistch, Human Anatomy, sixth ed. Pearson Education Inc., Toronto, 2008. 518–519 (685-686).
[2] J.-P. Hallé, P. de Vos, The Need for New Therapeutic Approaches and the Bioartificial Endocrine Pancreas, in: J.P. Hallé, P. de Vos, L. Rosenberg (Eds.), The Bioartificial Pancreas and Other Biohybrid Techniques, Transworld Research Network, Kerala, India, 2009, pp. 1–26.
[3] M. Perez-Basterrechea, R.M. Briones, M. Alvarez-Viejo, E. Garcia-Perez, M.M. Esteban, V. Garcia, A. Obaya, L. Barneo, A. Meana, J. Otero, Plasma–fibroblast gel as scaffold for islet transplantation, Tissue Eng. A 15 (3) (2009) 569–577.
[4] R.B. Elliot, L. Escobar, P.L.J. 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.
[5] D. Brendenburg, History and diagnostic significance of C-peptide, Exp. Diabetes Res. 2008 (576862) (2008) 1–7, http://dx.doi.org/10.1155/2008/576862 (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2396242).
[6] M.E. Williams, E. Lacson, M. Teng, N. Ofsthun, J.M. Lazarus, Hemodialyzed type I and type II diabetic patients in the US: characteristics, glycemic control, and survival, Kidney Int. 70 (2006) 1503–1509.
[7] Canadian Diabetes Association, Diabetes: Canada at the Tipping Point, http://www.diabetes.ca/documents/get-involved/WEB_Eng.CDA_Report_.pdf. April 2011.
[8] M.C. Skarulis, B. Hirshberg, Hypoglycemia in the Adult, in: D. LeRoith, S.I. Taylor, J.M. Olefski (Eds.), Diabetes Mellitus: A Fundamental and Clinical Text, Lippincott Williams & Wilkins, Philadelphia, 2004, pp. 1427–1438.
[9] N. Kobayashi, Bioartificial pancreas for the treatment of diabetes, Cell Trans. 17 (2008) 11–17.
[10] The Diabetes Control and Complications Trial Research Group, The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus, N. Engl. J. Med. 329 (1993) 977–986.
[11] Canadian Diabetes Association, The Prevalence and Cost of Diabetes, April 2008. (http://www.diabetes.ca/Files/prevalence-and-costs.pdf. Accessed May 24, 2011).
[12] P.W. Williams, Notes on diabetes treated with extract and by grafts of sheep's pancreas, BMJ 2 (1894) 1303–1304.
[13] A. Gruessner, Update on pancreas transplantation: comprehensive trend analysis of 25,000 cases followed up over the course of twenty-four years at the international pancreas transplant registry (IPTR), Rev. Diabetes Stud. 8 (2011) 6–16.
[14] S.A. White, J.A. Shaw, D.E.R. Sutherland, Pancreas transplantation, Lancet 373 (2009) 1808–1817.
[15] M.D. Stegall, P.G. Dean, R. Sung, The rationale for the new deceased donor pancreas allocation schema, Transplantation 83 (2007) 1156–1161.
[16] P.E. Lacy, M. Kostianovsky, Method for the isolation of intact islets of Langerhans from the rat pancreas, Diabetes 16 (1967) 35–39.
[17] D. Bosco, P. Meda, Reconstructing islet function in vitro, Adv. Exp. Med. Biol. 426 (1997) 285–298.
[18] A.M.J. Shapiro, J.R.T. Lakey, E.A. Ryan, G.S. Korbutt, E. Toth, G.L. Warnock, N.M. Kneteman, R.V. Rajotte, Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen, NEJM 343 (2000) 230–238.
[19] Roche Diagnostics GmbH, Liberase, http://www.roche-applied-science.com. May, 2013.
[20] CITR (Collaborative Islet Transplant Registry), 2009 Annual Report, Dec 2009, (http://www.citregistry.org).
[21] 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, Emerg. Treat. Technol. 32 (2009) 1887–1889.
[22] D.W. Scharp, P.E. Lacy, J.V. Santiago, C.S. McCullough, L.G. Weide, L. Falqui, P. Marchetti, R.L. Gingerich, A.S. Jaffe, P.E. Cryer, Insulin independence after islet transplantation into type I diabetic patient, Diabetes 39 (1990) 515–518.
[23] CITR (Collaborative Islet Transplant Registry), Seventh Annual Report, Dec 2011, (http://www.citregistry.org).
[24] A.M.J. Shapiro, C. Ricordi, B.J. Hering, H. Auchincloss, R. Lindblad, P. Robertson, A. Secchi, M.D. Brendel, T. Berney, D.C. Brennan, E. Cagliero, International trial of the Edmonton protocol for islet transplantation, NEJM 355 (2006) 1318–1330.
[25] CITR (Collaborative Islet Transplant Registry), Fifth Annual Report, Sept 2008, (http://www.citregistry.org).
[26] A.M.J. Shapiro, H.L. Gallant, E.G. Hao, J.R.T. Lakey, T. McCready, R.V. Rajotte, R.W. Yatscoff, N.M. Kneteman, The portal immunosuppressive storm — relevance to islet transplantation, Ther. Drug Monit. 27 (2005) 35–37.
[27] T. Eich, O. Eriksson, A. Sundin A, Positron emission topography: a real time tool to quantify early islet engraftment in a preclinical large animal model, Transplantation 84 (2007) 893–898.
[28] L. Jansson, P.O. Carlsson, Graft vascular function after transplantation of pancreatic islets, Diabetologia 45 (2002) 749–755.
[29] A.M. Hiscox, A.L. Stone, S. Limesand, J.B. Hoying, S.K. Williams, An islet-stabilizing implant constructed using a preformed vasculature, Tissue Eng. A 14 (2008) 433–440.
[30] Y. Liu, M. Petreaca, M. Martins-Green, Cell and molecular mechanisms of insulin-induced angiogenesis, J. Cell. Mol. Med. 12 (2008) 1–13.
[31] U.A. Stock, J.P. Vacanti, Tissue engineering: current state and prospects, Annu. Rev. Med. 52 (2001) 443–450.
[32] B. Amory, J.L. Mourmeaux, C. Remacle, *In vitro* cytodifferentiation of perinatal rat islet cells within a tridimensional matrix of collagen, In Vitro Cell. Dev. Biol. 24 (1988) 91–96.

[33] M.D. Brendel, S.S. Kong, R. Alejandro, D.J. Mintz, Improved functional survival of human islets of Langerhans in three-dimensional matrix culture, Cell Transplant 3 (1994) 427–433.
[34] G.M. Beattie, A.M. Montgomery, A.D. Lopez, E. Hao, B. Perez, M.L. Just, J.R. Lakey, M.E. Hart, A. Hayek, A novel approach to increase human islet cell mass while preserving beta-cell function, Diabetes 51 (2002) 3435–3439.
[35] A. Hyder, C. Laue, J. Schrezenmeir, Effect of the immunosuppressive regime of Edmonton protocol on the long-term in vitro insulin secretion from islets of two different species and age categories, Toxicol. in Vitro 19 (2005) 541–546.
[36] F. Lim, A.M. Sun AM, Microencapsulated islets as bioartificial endocrine pancreas, Science 210 (1980) 908–910.
[37] G. Orive, R.M. Hernandez, A.R. Gascon, R. Calafiore, T.M.S. Chang, P. de Vos, G. Hortelano, D. Hunkeler, I. Lacik, J.L. Pedraz, History, challenges and perspectives of cell microencapsulation, Trends Biotechnol. 22 (2004) 87–92.
[38] H. Zimmermann, D. Zimmermann, R. Reuss, P.J. Feilen, B. Manz, A. Katsen, M. Weber, F.R. Ihmig, F. Ehrhart, P. Geßner, M. Behringer, Towards a medically approved technology for alginate based microcapsules allowing long term immunoisolated transplantation, J. Mater. Sci. 16 (2005) 491–501.
[39] A. Omer, V. Duvivier-Kali, J. Fernandes, Long-term normoglycemia in rats receiving transplants with encapsulated islets, Transplantation 79 (2005) 52–58.
[40] C.A. Hoesli, R.L.J. Kiang, D. Mocinecova, M. Speck, D. Jochec Moskova, C. Donald-Hague, I. Lacik, T.J. Kieffer, J.M. Piret, Reversal of diabetes by βTC3 cells encapsulated in alginate beads generated by emulsion and internal gelation, J. Biomed. Mater. Res. B Appl. Biomater. 100B (2012) 1017–1028.
[41] P. de Vos, M.M. Faas, B. Strand, R. Calafiore, Alginate-based microcapsules for immunoisolation of pancreatic islets, Biomathematics 27 (2006) 5603–5617.
[42] V. Dugas, C. Beauchamp, J.P. Hallé, S. Lesage, Protection by microencapsulation from antigen recognition, immune cells, antibodies, and compliment components, in: J.P. Hallé, P. de Vos, L. Rosenberg (Eds.), The Bioartificial Pancreas and Other Biohybrid Techniques, Transworld Research Network, Kerala, India, 2009, pp. 261–277.
[43] T.M.S. Chang, Semipermeable microcapsules, Science (1964) 524–525.
[44] P. de Vos, A. Andersson, S.K. Tam, M. Fass, J.P. Hallé, Advances and barriers in mammalian cell encapsulation for treatment of diabetes, Immunol. Endocrinol. Metab. Agents Med. Chem. 6 (2006) 139–153.
[45] R. Robitaille, J. Dusseault, N. Henley, L. Rosenberg, J.P. Hallé, Insulin-like growth factor II allows prolonged blood glucose normalization with a reduced islet cell mass transplantation, Endocrinology 144 (2003) 3037–3045.
[46] P. de Vos, M.M. Faas, B. Strand, R. Calafiore, Alginate-based microcapsules for immunoisolation of pancreatic islets, Biomathematics 27 (2006) 5603–5617.
[47] T. Zekorn, U. Siebers, A. Horcher, R. Schnettler, G. Klöck, R.G. Bretzel, U. Zimmermann, K. Federlin, Barium-alginate beads for immunoisolated transplantation of islets of Langerhans, Trans. Proc. 24 (1992) 937–939.
[48] W.E. Mueller-Klieser, R.M. Sutherland, Influence of convection in the growth medium on oxygen tensions in multicellular tumor spheroids, Cancer Res. 42 (1982) 237–242.
[49] D. Poncelet, S.K. Tam, Microencapsulation Technologies for a Bioartifical Endocrine Pancreas, in: J.P. Hallé, P. de Vos, L. Rosenberg (Eds.), The Bioartificial Pancreas and Other Biohybrid Techniques, Transworld Research Network, Kerala, India, 2009, pp. 37–50.
[50] D. Poncelet, R.J. Neufeld, M.F.A. Goosen, B. Burgarski, V. Babak, Formation of microgel beads by electric dispersion of polymer solutions, AIChE J 45 (1999) 2018–2023.
[51] D. Serp, E. Cantana, C. Heinzen, U. von Stockar, I.W. Marison, Characterization of an encapsulation device for the production of monodisperse alginate beads for cell immobilization, Biotechnol. Bioeng. 70 (2000) 41–53.
[52] U. Prusse, P. Jahnz, J. Wittlich, J. Breford, K.D Vorlop, Bead Production with JetCutting and Rotating Disc/Nozzle Technologies, in: U. Prusse, K.D. Vorlop (Eds.), Practical aspects of encapsulation technologies, Bundesforschungsanstalt Landwirtschaft, Braunschweig, Germany, 2002, pp. 1–10.
[53] J.W. Strutt, J.W.S. Rayleigh, On the instability of jets, 1878, Proc. Lond. Math. Soc. 10 (1878) 4–13.
[54] D. Poncelet, R. Lencki, C. Beaulie, R.J. Neufeld, Production of alginate beads by emulsification/internal gelation. I. Methodology, Appl. Microbiol. Biotechnol. 38 (1992) 39–45.
[55] D. Poncelet, B. Poncelet De Smet, B, C. Beaulieu, M.L. Huguet, A. Fournier, R.J. Neufeld. Production of alginate beads by emulsion/internal gelation. II. Physicochemistry, Appl. Microbiol. Biotechnol. 43 (1995) 644–650.
[56] C.A. Hoesli, K. Raghuram, R.L.J. Kiang, D. Mocinecova, X. Hu, J.D. Johnson, I. Lacik, T.J. Kieffer, J.M. Piret, Pancreatic cell immobilization in alginate beads produced by emulsion and internal gelation, Biotechnol. Bioeng. 108 (2011) 424–434.
[57] R. Robitaille, J.P. Hallé, Characterisation and Evaluation of the Properties and Functions of the Microcapsules, in: J.P. Hallé, P. de Vos, L. Rosenberg (Eds.), The Bioartificial Pancreas and Other Biohybrid Techniques, Transworld Research Network, Kerala, India, 2009, pp. 67–85.
[58] B. Thu, G. SkjakBraek, F. Micali, F. Vittur, R. Rizzo, The spatial distribution of calcium in alginate gel beads analysed by synchrotronradiation induced X-ray emission (SRIXE), Carbohydr. Res. 297 (1997) 101–105.
[59] J.Y.C. Cheng, M. Rughunath, J. Whitelock, L. Poole-Warren, Matrix components and scaffolds for sustained islet function, Tissue Eng. B 17 (2011) 235–247.
[60] L.M. Weber, K.N. Hayda, K.S. Anseth, Cell-matrix interactions improve β-cell survival and insulin secretion in three-dimensional culture, Tissue Eng. A 14 (2008) 1959–1967.
[61] J. Daoud, M. Petropavlovskaia, L. Rosenberg, M. Tabrizian, The effect of extracellular matrix components on the preservation of human islet function in vitro, Biomaterials 31 (2010) 1676–1682.
[62] C. Del Guerra, K. Bracci, Nilsson, entrapment of dispersed pancreatic islet cells in CultiSpher-S macroporous gelatin microcarriers, Biotechnol. Bioeng. 75 (2001) 741–744.
[63] R. Landers, A. Pfister, U. Hubner, R. Schmelzeisen, R. Mulhaupt, Fabrication of Soft Tissue Engineering Scaffolds by Means of Rapid Prototyping Techniques, 372002. 3107–3116.
[64] C. Lucas-Clerc, C. Massart, J.P. Campion, Long-term culture of human pancreatic islets in an extracellular matrix: morphological and metabolic effects, Mol. Cell. Endocrinol. 94 (1993) 9–14.
[65] S. Chun, Y. Huang, W.J. Xiw, Adhesive growth of pancreatic islet cells on a PGA fibrous scaffold, Trans. Proc. 40 (2008) 1658–1663.
[66] D.M. Salvay, C.B. Rives, X. Zhang, F. Chen, D.B. Kaufman, W.L. Lowe, L.D. Shea, Extracellular matrix protein-coated scaffolds promote the reversal of diabetes after extrahepatic islet transplantation, Transplantation 85 (2008) 1456–1464.
[67] Y. Hou, C. Song, W.J. Xie, Z. Wei, R.P. Huang, W. Liu, Z.L. Zhang, Y.B. Shi, Excellent effect of three-dimensional culture condition on pancreatic islets, Diabetes Res. Clin. Pract. 86 (2009) 11–15.
[68] N. Kawazoe, X. Lin, T. Tateishi, Three-dimensional cultures of rat pancreatic RIN-5 F cells in porous PLGA–collagen hybrid scaffolds, J. Bioact. Compat. Polym. 24 (2009) 25–42.
[69] F.X. Jiang, G. Naselli, L.C. Harrison, Distinct distribution of laminin and its integrin receptors in the pancreas, J. Histochem. Cytochem. 50 (2002) 1625–1632.
[70] Y. Hamamoto, S. Fujimoto, A. Inada, Beneficial effect of pretreatment of islets with fibronectin on glucose tolerance after islet transplantation, Horm. Metab. Res. 35 (2003) 460–465.
[71] T. Kaido, M. Yebra, V. Cirulli, Regulation of human beta-cell adhesion, motility, and insulin secretion by collagen IV and its receptor alpha1 beta1, J. Biol. Chem. 279 (2004) 53762–53769.
[72] J.T. Daoud, M.S. Petropavlovskaia, J.M. Patapas, C.E. Degrandpré, R.W. DiRaddo, L. Rosenberg, M. Tabrizian, Long-term *in vitro* human pancreatic islet culture using three-dimensional microfabricated scaffolds, Biomaterials 32 (2011) 1536–1542.
[73] J.A.M. Steele, A.E. Barron, E. Carmona, J.-P. Hallé, R.J. Neufeld, Encapsulation of protein microfiber networks supporting pancreatic islets, J. Biomater. Mater. Res. A 100 (2012) 3384–3391.
[74] O. Smidsrød, G. Skjåk-Braek, Alginate as immobilization matrix for cells, Trends Biotechnol. 8 (1990) 71–78.
[75] A.G. Mallet, G.S. Korbutt, Alginate modification improves long-term survival and function of transplanted encapsulated islets, Tissue Eng. A 15 (2009) 1301–1309.
[76] P. de Vos, M. Bucko, P. Gemeiner, M. Navratil, J. Svitel, M. Faas, B.L. Strand, G. Skjak-Braek, Y.A. Morch, A. Vikartovska, I. Lacik, G. Kollarikova, G. Orive, D. Poncelet, J.L. Pedraz, M.B. Ansorge-Schumacher, Multiscale requirements for bioencapsulation in medicine and biotechnology, Biomaterials 30 (2009) 2559–2570.
[77] 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.
[78] M. Ménard, J. Dusseault, G. Langlois, W.E. Baille, S.K. Tam, L.H. Yahia, X.X. Zhu, J.P. Hallé, Role of protein contaminants in the immunogenicity of alginates, J. Biomed. Mater. Res. B 93 (2010) 333–340.
[79] A.P. Monaco, T. Maki, H. Ozato, M. Carretta, S.J. Sullivan, K.M. Borland, M.D. Mahoney, W.L. Chick, T.E. Muller, J. Wolfrum, B. Solomon, Transplantation of islet allografts and xenografts in totally pancreatectomized diabetic dogs using the hybrid artificial pancreas, Ann. Surg. 214 (1991) 339–362.
[80] P. de Vos, B.J. de Haan, M.M. Faas, Different Approaches for Immunoisolation, in: J.P. Hallé, P. de Vos, L. Rosenberg (Eds.), The Bioartificial Pancreas and Other Biohybrid Techniques, Transworld Research Network, Kerala, India, 2009, pp. 27–36.
[81] S. Sandler, D.L. Eizirik, J. Sternesjo, N. Welsh, Role cytokines in regulation of pancreatic b-cell function, Biochem. Soc. Trans. 22 (1994) 26–30.
[82] F.A. Leblond, G. Simard, N. Henley, B. Rocheleau, P.M. Huet, J.P. Hallé, Studies on smaller (similar to 315 μm) microcapsules: IV feasibility and safety of intrahepatic implantations of small alginate poly-L-lysine microcapsules, Cell Transplant. 8 (1999) 327–337.
[83] P. de Vos, A.F. Hamel, K. Tatarkiewicz, Considerations for successful transplantation of encapsulated pancreatic islets, Diabetologia 45 (2002) 159–173.
[84] S. Muthyala, V.R.R. Raj, M. Mohanty, P.V. Mohanan, P.D. Nair, The reversal of diabetes in rat model using mouse insulin producing cells — a combination approach of tissue engineering and macroencapsulation, Acta Biomater. 7 (2011) 2153–2162.
[85] A. Townsend-Nicholson, S.N. Jayasinghe, Cell electrospinning: a unique biotechnique for encapsulating living organisms for generating active biological microthreads/scaffolds, Biomacromology 7 (2006) 3364–3369.
[86] H. Onoe, T. Okitsu, A. Itou, M. Kato-Negishi, R. Gojo, D. Kiriya, K. Sato, S. Miura, S. Iwanaga, K. Kuribayashi-Shigetomi, Y.T. Matsunaga, Y. Shimoyama, S. Takeuchi, Metre-long cell-laden microfibers exhibit tissue morphologies and functions, Nat. Mater. 12 (2013) 584–590.
[87] R. Calafiore, G. Basta, G. Luca, A. Lemmi, M.P. Montanucci, G. Calabrese, L. Racanicchi, F. Manusco, P. Brunetti, Microencapsulated pancreatic islet allografts into nonimmunosuppressed patients with type 1 diabetes, Emerg. Treat. Technol. 29 (2006) 137–138.
[88] R. Calafiore, G. Basta, G. Luca, A. Lemmi, M.P. Montanucci, L. Racanicchi, F. Manusco, P. Brunetti, Standard technical procedures for microencapsulation of human islets for graft into nonimmunosuppressed patients with type 1 diabetes mellitus, Transplant. Proc. 38 (2006) 1156–1157.
[89] http://clinicaltrials.gov/ct2/show/NCT00940173.
[90] http://www.lctglobal.com/Products-and-Services/Diabecell.
[91] http://viacyte.com/products/vc-01-diabetes-therapy.
[92] http://clinicaltrials.gov/ct2/show/NCT00260234.
[93] http://www.prnewswire.com/news-releases/novocell-becomes-viacyte-inc-as-it-accelerates-pre-clinical-development-of-a-stem-cell-derived-treatment-for-diabetes-92923104.html.
[94] http://clinicaltrials.gov/ct2/show/record/NCT01379729.
[95] http://clinicaltrials.gov/ct2/show/NCT00790257.