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Teaser The most-recently developed strategies for islet encapsulation are reviewed regarding type 1 diabetes mellitus treatment aiming at efficient insulin production and delivery.

Paving the way for successful islet encapsulation

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Type 1 diabetes mellitus (T1DM) is a disorder that decimates pancreatic β-cells which produce insulin. Direct pancreatic islet transplantation cannot serve as a widespread therapeutic modality owing to the need for lifelong immunosuppression and donor shortage. Therefore, several encapsulation techniques have been developed to enclose the islets in semipermeable vehicles that will allow oxygen and nutrient input as well as insulin, other metabolites and waste output, while accomplishing immunoisolation. Although encapsulation technology continues to face significant obstacles, recent advances in material science, stem cell biology and immunology potentially serve as pathways to success. This review summarizes the accomplishments of the past 5 years.

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Introduction

The pancreas is a glandular organ of the digestive and endocrine systems. It has lobules, which are formed by a mixture of ductules and well-vascularized epithelial cell clusters that reflect the two main functions of the pancreas: digestion and glucose homeostasis. Exocrine cells, which form the majority of the parenchyma, release a mixture of digestive enzymes and bicarbonate into the duodenum. The remaining 1–2% of the pancreatic mass consists of endocrine cells, which form glands and are called islets of Langerhans. These islets consist of five cell types: β-cells, α-cells, δ-cells, ε-cells and pancreatic polypeptide cells. The most important cell type in regulating glucose metabolism is the β-cell, which represents ~65–80% of the total islet population. β-cells secrete the body's insulin, which is directly related to daily carbohydrate intake and physical activity. Insulin stimulation occurs in the event of high plasma glucose, glucagon, parasympathetic stimulation, glucagon-like peptide 1 (GLP-1), glucose-dependent insulinotropic peptide (GIP) and secretion of other peptides [1].

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Islet β-cells exhibit more-potent secretory responses than dissociated cells. The molecular and cellular basis of this connectivity is affected by a series of diabetic insults and genes associated with diabetes. However, owing to certain factors (genetic, environmental, immunological) in one class of people no insulin is produced [2]. In this event, a malady called type 1 diabetes mellitus (T1DM) occurs, and patients who develop the condition are called insulin-dependent. T1DM results from complete destruction of endocrine pancreatic β-cells, resulting in insulin deficiency [2]. β-cell destruction precedes the onset of symptoms by several months or years when the patients are asymptomatic and euglycemic but present positively related antibodies. In this regard, diabetes occurs after a long latency period, correlated with the high percentage of β-cells, which must be destroyed to manifest the disease.

Currently, the main treatment for T1DM is exogenous insulin therapy via injections. Islet transplantation with immunosuppression, known as the Edmonton protocol, has been proposed as a therapeutic modality for T1DM treatment. Transplanting pancreatic islets has important advantages over transplanting the whole organ, because it involves a minor surgical procedure with low morbidity and mortality. In clinical islet transplantation, the required amount of islet equivalents (IEQ; i.e., islets with mass equal to that of an islet of average size, 150 μm) is associated with the recipient's body weight (BW). Most clinical protocols determine the lower threshold for human islet transplantation as 5000 IEQ/kg BW, whereas trials with larger cohorts of patients raise the number up to 10 000 IEQ/kg BW [3]. In allotransplantation there is a limited supply of human pancreata; however, recent progress in the stem cell field can potentially eliminate this donor shortage barrier [4].

Immunoisolation is proposed for the obviation of immunosuppressive drugs that are associated with recurrent side effects. Islets are encapsulated in a barrier vehicle, which protects the islets against host immune responses, while simultaneously facilitating the exchange of vital molecules, such as oxygen, nutrients and insulin. The material needs to be biocompatible, yet not biodegradable, facilitate neovascularization and exchange of vital molecules, and prevent invasion of toxic chemical and cellular moieties (antibodies, cytokines, etc.). In this review, the accomplishments from the past 5 years of islet encapsulation technologies and approaches to overcome important limitations are epitomized.

Encapsulation strategies

Strategies for the encapsulation of pancreatic islets can be grouped into three main categories: (i) nanoencapsulation, (ii) microencapsulation and (iii) macroencapsulation (Fig. 1). The presented classification is made by considering the mean distance of the islets from the host environment, as the most significant parameter of cell viability and function. Nano-, micro- and macro-encapsulation paradigms are demonstrated in Tables 1 and 2.

Nanoencapsulation

Layer-by-layer (LbL) deposition of oppositely charged biomaterials is the most commonly used method for islet nanoencapsulation. Nanoscale LbL coatings were developed expressing bio-orthogonal functionality and tailored physicochemical properties. A recent study established an encapsulation method based on LbL assembly that facilitates the cell surface to be coated with extracellular matrix (ECM). This technique enabled the rapid fabrication of well-structured β-cell spheroids enhancing intracellular interaction and its effectiveness was tested in a mouse model [5].

A xenotransplantation of LbL non-human primate (NHP) islets was conducted by Haque *et al*. to test a three-polymer LbL encapsulation of NHP islets in mice with immunosuppression. They claimed that the LbL encapsulation showed a uniform nanoshielding on islets without the loss of viability and function [6]. Recently, Park and co-workers transplanted heparin nanoshielded islets (10000 IEQ/kg BW) into NHPs with immunosuppressive drugs that reduced instant blood-mediated inflammatory reactions (IBMIR) [7]. Additionally, an ultrathin heparin-polymer nanofilm was proposed by another group, providing an open platform that enables incorporation of biological mediators for islet surface bioengineering [8].

Microencapsulation

The microencapsulation approach indicates the encapsulation of individual or small clusters of islets. Microcapsules are the most common microencapsulation vehicles and have been broadly investigated and tested in several animal models. Ma and co-workers encapsulated cells in alginate-based hydrogel microcapsules with core–shell structures using two-fluid coaxial electrojetting, which provided better treatment than conventional capsules in a mouse model [9]. Furthermore, Teramura *et al*. proposed a microencapsulation method within an ultrathin polymer membrane (~10 μm) that is thicker than conventional LbL membranes and thus more stable [10].

Tomei and co-workers developed a strategy to minimize the distance from islet to the host tissue by exploiting an optimized hydrodynamic focusing technique [11]. The so-called conformal coating allows complete microencapsulation with a thin layer (10–50 μm) of hydrogel that secures islet function in a rodent model. The same group later produced poly(ethylene glycol) (PEG)–maleimide Matrigel® (islet-like ECM) which encapsulated islets via conformal coating and presented long-term survival after allotransplantation in the epididymal fat pad (EFP) site (750–1000 IEQ/mouse) in the absence of immunosuppression – no T cell activation was observed [12]. Furthermore, an encapsulation platform based on pancreatic ECM was developed by Chaimov *et al*. and was assessed in a proof-of-concept study using a mouse model. Specifically, ECM-encapsulated cells were shown to be nonimmunogenic and glycemic control was improved [13].

Macroencapsulation

The main distinction of macroencapsulation devices is the housing of a large mass of islets. Depending on the transplantation site, macrodevices can be divided into two categories: extravascular and intravascular. The encapsulation devices are transplanted extrahepatically; however, in this case the rapid angiogenesis is of crucial importance for graft survival. Subcutaneous transplantation facilitates graft retrieval and monitoring [14,15]. Extravascular macrocapsules, owing to a lower surface:volume ratio compared with microcapsules, need more space and a transplantation site with established vascular beds. Therefore, such devices are often placed in the peritoneal cavity from which the device can later be explanted or retrieved.

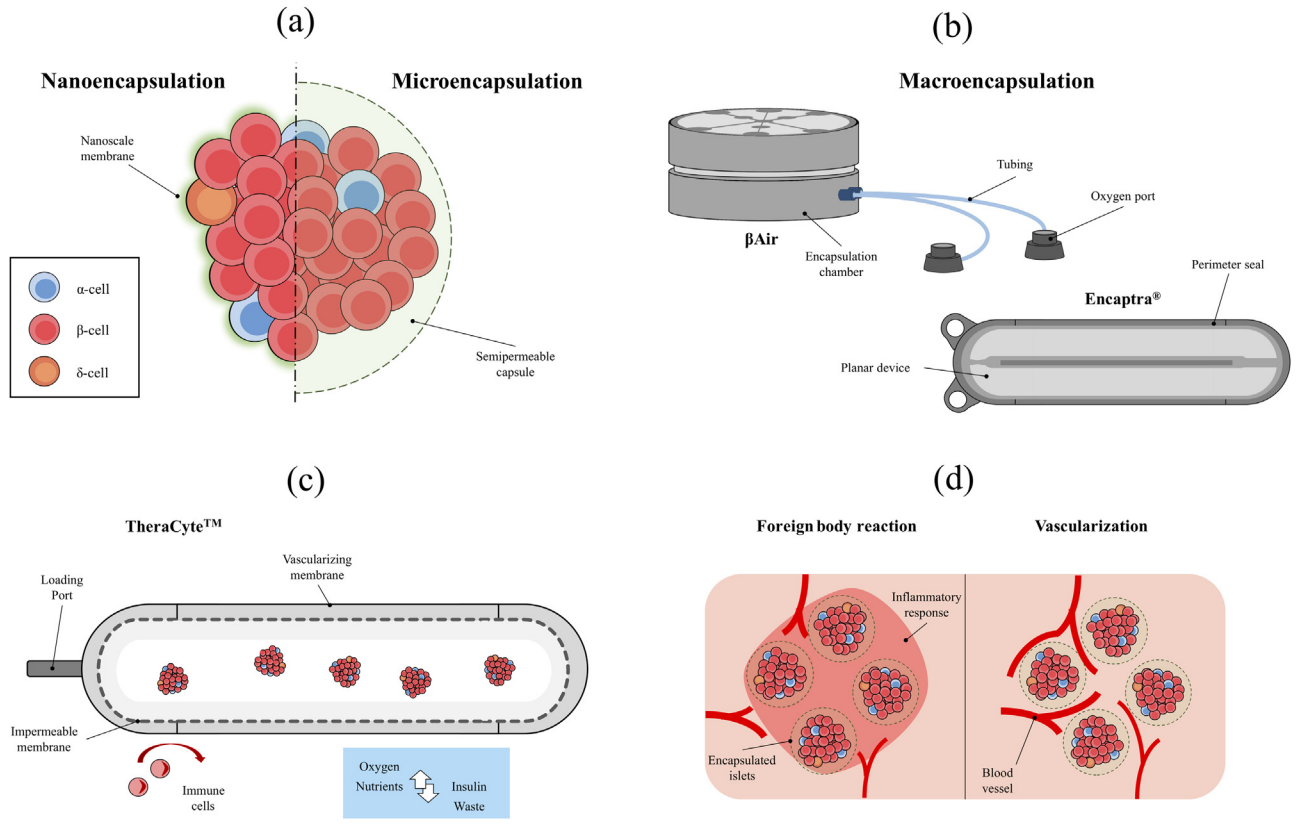

FIGURE 1

Schematic illustration of pancreatic islet encapsulation. **(a)** Nanoencapsulation and microencapsulation of an islet. **(b)** Macroencapsulation devices house a large mass of islets. Encaptra® is a planar device containing a semipermeable barrier, whereas βAir is a device with ports for recharging oxygen. **(c)** Immunosuppression using TheraCyte™, a thin biocompatible membrane-bound polymeric chamber. **(d)** Shortly after the transplantation a host response occurs. Over time, a vascular network forms providing the supply of oxygen and nutrients for the encapsulated islets.

TABLE 1

***In vivo* nano- and micro-encapsulation studies**

	Material	Cell type/host	Site	Description	Refs
Nano	PEG and heparin	NHP/NHP	Portal vein	Nano-shield, LbL approach	[7]
	Fibronectin and gelatin	Mice/mice	Intraperitoneal cavity	Nanofilms on cell surface, LbL approach	[5]
	PEG molecules	NHP/mice	Kidney	Three layers of SH-6-arm-PEG-NHS, 6-arm-PEG-catechol and linear PEG-SH	[6]
	PEG-MAL MG DTT	Mice/mice	EFP	Conformal coating, PEG and islet- like ECM (Matrigel®; MG) islet encapsulation	[12]
	Polyphenol nanothin coatings	Mice/mice	EFP	Multilayer coating consisting of tannic acid, an immunomodulatory antioxidant and PVPON	[88]
Micro	Alginate-AEMA	Mice/rat	Omentum	Alginate with 2-aminoethyl methacrylate hydrochloride, photocrosslinking	[33]
	PFD-alginate	Mice/Mice	Peritoneal cavity	Suspension of islets in polymer solution, coaxial bead generator	[70]
	ECM	hESC/Mice	Subcutaneous	ECM composed of polysaccharides and proteins, mainly collagen and elastin	[13]
	Chitosan-coated alginate	Pig, dog/mice, dog	Intraperitoneal cavity	Alginate capsules suspended in chitosan solution, air-driven droplet generator	[38]
	PEG	Mice/mice	Kidney	Capsule, conformal coating of islets through microfluidics	[11]
	Alginate	Rat/mice	Peritoneal cavity	Core-shell capsule,co-axial nozzle for a stable formation of uniform core–shell	[9]

Abbreviation: PEG, polyethylene glycol; NHP, non-human primate; LbL, layer by layer; NHS, *N*-hydroxysuccinimide; MAL, maleimide; DTT, dithiothreitol; EFP, epididymal fat pad; ECM, extracellular matrix; PVPON, poly(N-vinylpyrrolidone); AEMA, 2-aminoethyl methacrylate hydrochloride; PFD, perfluorodecalin; hESC, human embryonic stem cells.

TABLE 2

***In vivo* macroencapsulation studies: methods and devices**

Method or device	Cell type/host	Site	Description	Refs
βAir	Rat/rat	Subcutaneous	([a])	[17]
TRAFFIC	Rat, human/mice	Peritoneal cavity	Ca^{2+}-releasing nanoporous polymer thread that promoted uniform *in situ* crosslinking and strong adhesion of a thin layer of alginate hydrogel around the thread	[31]
Device-encapsulated hESC-PE	hESC/mice	Subcutaneous	hESC-derived pancreatic endoderm, engineered Encaptra® drug delivery system	[21]
3D printed PLA	Human/mice	Kidney	PLA discoidal encapsulation devices – 8 mm in diameter and 2.5 mm in thickness	[27]
SNM-based iBAP	Mice/pig	Intravascular	Intravascular BAP with ~10 and ~40 nm pore-sized membranes	[29]
βAir	Pig/NHP	Between the parietal peritoneum and the fascia of the abdominal muscles	([a])	[16]
PDLLCL scaffold	Rat/rat	Subcutaneous	Scaffold transplantation to initiate vascularization	[75]
Biologic scaffold	Rat, NHP, human/rat, NHP	Intraomental	Plasma and recombinant human thrombin	[28]
3D printed PLA scaffold	hESC/mice	Subcutaneous	Macroporous PLA with fibrin gel	[25]
TheraCyte™	Mice/mice	Subcutaneous	([b])	[18]
PEG	Mice/mice	EFP	Combines encapsulation with lithography techniques to generate PDMS molds, 600 mm thickness	[100]
3D printed alginate scaffold	Rat	Subcutaneous	Alginate/gelatin blend	[26]
PCL thin-film device	Mice/mice	Under the abdomen above the liver or subcutaneous	PCL micro- and nano-porous thin-films heat-sealed together using resistive heating of a nichrome wire	[23]
Encaptra®	hESC/mice	Subcutaneous	PEC-01 cell population, differentiated from hESCs, contains pancreatic progenitors	[20]
TheraCyte™	hESC/mice	Subcutaneous	([b])	[15]
NanoGland	Human/mice	Subcutaneous	Microfabricated silicon membrane with parallel nanochannels (3.6–40 nm) and vertical microchannels (20–60 mm)	[22]
PDMS scaffold	Rodent, NHP, human/rat	Omentum	Biostable, macroporous scaffolds, fabricated using solvent casting and particulate leaching technique	[101]
TheraCyte™	Rat/rat	Subcutaneous	([b])	[19]
VEGF-PEG-MAL	Mice/mice	Peritoneal cavity	Polymer and islets mixed then crosslinked	[68]

Abbreviation: hESC-PE, human embryonic stem cell-derived pancreatic endoderm; PLA, polylactic acid; SNM, silicon nanopore membranes; iBAP, intravascular bioartificial pancreas device; NHP, non-human primate; PDLLCL, poly(D,L-lactide-co-e-caprolactone); PEG, polyethylene glycol; EFP, epididymal fat pad; PDMS, poly(dimethylsiloxane); PCL, polycaprolactone; VEGF, vascular endothelial growth factor; MAL, maleimide.

[a] A chamber, 68 mm in diameter and 18 mm in thickness, composed of a 600-μm-thick immobilized islets module attached to an oxygen module by a gas-permeable silicon-rubber membrane.

[b] Thin bound polymeric chamber composed of two membranes.

Macroencapsulation devices have already been commercialized, such as Theracyte™, βAir from BetaO2 Technologies, and Encaptra® of ViaCyte. The βAir device consists of three major compartments: (i) an islet compartment with islets embedded in a 500–600 μm thick alginate slab; (ii) a gas chamber pO2 with inlet and outlet ports; and (iii) a 25 μm polytetrafluoroethylene (PTFE) membrane serrating the islet module from the host tissue. A newly successful and safe xenotransplantation of porcine islets in a diabetic NHP without immunosuppression has been accomplished with favorable results [16]. Moreover, Evron and co-workers recently determined a supraphysiological level of the gas chamber pO2 to substantially increase islet surface density [17]. Another macroencapsulated system, the TheraCyte™ device, is a thin biocompatible membrane-bound polymeric chamber that has been tested in a large number of animal studies preventing hyperglycemia [18] and allograft rejection [19]. Encaptra® is a planar macroencapsulation device containing a semipermeable barrier that can be engineered properly and loaded with hESCs [20]. A newly published study determines the biologic characteristics at which this combination product, named VC-01™, establishes metabolic control in mouse models [21]. The silicon NanoGland macroencapsulation device has been developed for the autotransplantation of human islets. It consists of a microfabricated membrane with parallel nanochannels for immunoprotection and perpendicular microchannels that facilitate neovascularization. NanoGland xenograft of human islets was subcutaneously implanted in mice and lasted for >120 days [22].

Another endeavor includes the designing of a nanoporous thin-film device from polycaprolactone (PCL) that sustained viability of encapsulated islets in allogeneic mouse models for up to 3 months. Foreign-body response was prevented and rapid neovascularization around the device was facilitated [23]. Moreover, successful long-term animal studies were recently conducted and the results indicated evidence of engraftment, viability and function of cells encapsulated in the device after 6 months [24].

Biologic and bioengineered scaffolds enabled by 3D printing technology [25] are increasingly gaining attention as an alternative approach of islet macroencapsulation. Marchioli *et al.* constructed an alginate-based porous scaffold of a pre-defined 3D architecture and proceeded to transplantation into a mouse. However, impaired glucose diffusion and limited islet functionality were observed owing to the high viscosity of hydrogel needed for

bioplotting [26]. Farina and co-workers fabricated 3D-printed PLA encapsulation systems that generate vascularization in an immunocompromised mouse model [27]. Moreover, an *in-situ*-generated plasma–thrombin biologic scaffold was evaluated in rodent and NHP models with immunosuppression [28].

An intravascular bioartificial pancreas device (iBAP) was proposed as a macroencapsulation method that facilitates convective mass transport through silicon nanopore membranes (SNM). This device exhibited islet viability and functionality *in vitro*, providing insulin response to glucose stimulation for clinically relevant islet densities (5700 and 11 400 IE/cm^2). Subsequently, the SNM with ~10 nm pore size was applied in a porcine model where 85% islet viability was demonstrated at clinically relevant islet density (5700 IE/cm^2) and hemocompatibility through convection instead of diffusion [29]. This study could lead to scale-up BAP devices to be tested in larger animals and eventually T1DM patients.

A hydrogel-based, nanofiber-enabled encapsulation device (NEED) with macroscopic dimensions was proposed by An and co-workers as an alternative encapsulation strategy [30]. Requirements of biocompatibility and mechanical strength are fulfilled owing to the combination of hydrogel and nanofibers. The device was tested in a diabetic mouse and the diabetes was reserved for at least 8 weeks [30]. Furthermore, An and co-workers recently developed TRAFFIC (thread reinforced alginate fiber for islets encapsulation), a scalable and conveniently retrievable device for cell encapsulation [31]. Efficient function and translation are accomplished owing to uniform and controllable hydrogel and strong thread-hydrogel adhesion. The device provided immunoisolation of encapsulated rat and human islets in mouse models for 3 and 4 months, respectively.

New pathways to successful encapsulation

Material design considerations

The material used for encapsulation of islets or drugs could cause a host fibrotic response resulting in impaired mass transport in and out of the semipermeable membrane. Therefore, the choice of material is of great importance to modulate the host response and avoid graft rejection. This material should meet certain criteria such as biocompatibility, immunoisolation and semipermeability for nutrients and secreted metabolites or drug exchange. In general, natural polymers are preferred owing to the mild, nondestructive (to mammalian cells) conditions of gelling, with alginate as the most predominantly used owing to its production at neutral pH and mild temperatures [32,33]. Nevertheless, the batch-to-batch variability and composition divergence of β-D-mannuronic-acid:α-L-guluronic-acid ratio, as well as the residual components that the natural polymer could contain, such as endotoxins, polyphenols or proteins [34], compromise the islet viability because they elicit the host immune response and need to be encountered. Furthermore, the crosslinking ions and the presence or absence of additional polymer coating layers influence the success of cell encapsulation [32]. The increase of the guluronic-acid:mannuronic-acid ratio resulted in more-stable and biocompatible alginate structures as pinpointed in the literature [35]. Alginate hydrogels prepared via ion crosslinking, such as Ca^{2+}, present chemical instability after long-term application because of ion exchange between the divalent crosslinking ions and the monovalent ions, such as Na^+ existing in the surrounding environment. Somo and co-workers fabricated microbeads from methacrylated alginate, which enabled covalent crosslinking under exposure to ultraviolet light in addition to ionic interactions with divalent cations. Methacrylated alginate microbeads presented stability after 1 and 3 weeks under an inflammatory response in tests performed *in vivo,* using an omentum pouch model [33]. An alternative crosslinking procedure through 'click' chemistry has been described [36]. Alginate was functionalized to obtain pendant alkyne or azide functional groups, and subsequently 'click' gel capsules were formed with superior stability in ionic media and enhanced permeability to small diffusates. Another group introduced tetrazine and norbornene groups to the alginate backbone and then proceeded to 'click' crosslinking, resulting in more-stable structures that provided higher cell viability after encapsulation and minimal inflammatory response after injection in mice in comparison to ionically crosslinked gels that lost structural integrity and were infiltrated by fibroblasts and immune cells in a 1 month study [37].

In addition, combining alginate with other natural or synthetic materials has been examined for improved performance. Chitosan-coated alginate capsules were utilized for xenotransplantation of porcine islets in mice demonstrating a trend for graft survival with decreased pericapsular fibrosis and allotransplantation of canine islets in beagles where normoglycemia was maintained for 1 year [38]. Encapsulation of porcine islets was conducted in alginate capsules coated with methacrylated glycol chitosan via photopolymerization [39]. Silk macrocapsules have also been synthesized combined with alginate or agarose, with promising results of glucose-induced insulin secretion (GSIS) and immunomodulation, when tested *in vitro* [40]. Other natural materials that have been tested recently for cell encapsulation are ursodeoxycholic acid, agarose and hyaluronic acid and collagen [41,42].

Over the years, many researchers have focused on the use of synthetic polymers with reactive end groups that facilitate crosslinked configurations in mild conditions or a combination of natural and synthetic materials for cell encapsulation strategies, to avoid natural polymer limitations [43]. PEG is a synthetic polymer that can be functionalized with groups such as acrylates and methacrylates, enabling the formation of crosslinked bioinert hydrogel networks [43]. Marchioli and co-workers suggested islet encapsulation with a nonadhesive layer of PEG diacrylate (PEGDA). The authors described the synthesis of a bilayer with the combination of PEGDA with a second hydrogel based on thiolated-gelatin, thiolated-heparin and thiolated-hyaluronic-acid, which provided cues for endothelial cell adhesion. Alternatively, the same group supported the conformal coating of the islets with PEGDA and subsequent embedding in the pre-mentioned vascularization layer which is composed of thiolated glycosaminoglycans. This stable structure facilitates blood vessel ingrowth and maintains the round shape and functionality of the islets [44].

The volume of the implanted material and its configuration can influence the mass transfer and the islet survival. This was exemplified by Veiseh *et al.* who investigated the foreign body immune response of several materials, such as hydrogels, ceramics, metals and plastics, in rodents and NHP. They justified that encapsulation device biocompatibility is dependent on its size and shape [45].

The conformal coating of islets has proven an efficient strategy for islet immunoisolation, when the total size and volume of the encapsulation device is reduced [46]. In the literature, a light-mediated interfacial thiol-norbornene photopolymerization procedure was developed for creating hydrogel conformal coating on pancreatic islets with controlled coating thickness [47]. Tomei and

co-workers developed a strategy for the conformal coating of islets with PEG hydrogels of similar thickness through a flow-focusing technique followed by crosslinking [11]. The LbL nanocoating of NHP islets with three layers of PEG (SH-6-arm-PEG-NHS, 6-arm-PEG-catechol and linear PEG-SH) combined with an immunosuppressive drug protocol is described, achieving successful xenograft survival for 150 days [6]. In another case, LbL coating of PAMAM-MDT/alginate-hN3 was applied onto alginate microbeads resulting in stable structures, and their permselectivity could be regulated by the charge density of PAMAM, the number of layers and the length of the functional end groups [48].

In macroencapsulation devices, combinations of different materials with specific properties is essential for successful graft survival. The TRAFFIC device developed by An and co-workers consists of an alginate hydrogel layer strongly adhering to a tough nanoporous polymeric thread that includes nylon sutures coated with PMMA + Ca^{2+}. Thus, the device attained improved mechanical properties, whereas mass transfer was facilitated owing to the short diffusion distance [31]. βAir BAP of BetaO2 Technologies consists of two major separated components: an alginate hydrogel slab in which the islets are encapsulated and a gas chamber. The housing of the device is made of clinical-grade polyether ether ketone [49]. NEEDs are based on electrospun nanofibers impregnated with alginate, chitosan, collagen or PEG matrix [30]. The electrospun nanofibers attain small size (~10 nm to 10 μm), high porosity (>90%) and large surface area (~10 m^2/g), providing mechanical strength to the structure. The fiber material can alter their properties and the polymers nylon 6, polyacrylonitrile (PAN), PCL, polysulfone (PSU) and polystyrene (PS) have been tested by An *et al.* for designing nanofiber devices.

Ensuring cell supply

The limited donor availability renders human pancreatic cell supply infeasible because usually >2.5 human donors are required for a successful single transplantation. Taking this into consideration, the search for cell replacement strategies is mandatory. Over the years, allogeneic and xenogeneic cells have been successfully encapsulated and transplanted into rodents, dogs, NHPs and humans.

Stem cell therapy of T1DM represents a promising alternative strategy because stem cells have significant properties such as the ability to differentiate into glucose-responsive insulin-producing cells, along with their regenerative and immunomodulatory capacity. A variety of stem cells has been described in the literature for host normoglycemia restoration, including mesenchymal stem cells (MSCs) and pluripotent stem cells (PSCs), including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs).

MSCs present several advantages such as abundance and easy isolation from various tissues, including adipose tissue, bone marrow, menstrual blood, umbilical cord and dental pulp [50,51]. In the past, bone-marrow-derived MSCs differentiated into islet-like insulin-producing aggregates were encapsulated in a NanoGland device and cells maintained viability and function for at least 4 weeks with steady insulin production *in vitro*, justifying the potential of this cell source for diabetes therapy [22]. Recently, MSC islet allografts where PEGylated using *N*-hydroxysuccinimide (NHS)-PEG-CH_3. The NHS group facilitated reactivity to free amines, whereas the methyl group provided an inert terminal end. Subsequently, the islets were allotransplanted in mice and a synergistic relation was evaluated with the antilymphocyte function-associated antigen 1 (LFA-1) antibody, which was selected as a transient, systemic immune monotherapy, resulting in long-term euglycemia [52]. Research on MSCs is thoroughly described in a meticulous review by Dang *et al.* [53].

Human ESCs have the ability to regenerate and differentiate to insulin-producing cells when cultured in the appropriate microenvironment. Encapsulation of ESCs has been studied extensively in the literature. Vegas and co-workers reported the first long-term glycemic correction in diabetic, immune-competent mice, after encapsulation of hESC-β-cells in triazole-thiomorpholine dioxide (TMTD) alginate and intraperitoneal implantation, until removal after 174 days without any immunosuppression [54]. Kirk *et al.* proceeded with the encapsulation of islet progenitors derived from CyT49 hESCs in a PTFE bilaminar device (TheraCyte™) and proved immunoisolation in rodents for up to 150 days, until termination; however, sufficient insulin levels were achieved by 20-weeks post-transplant [15]. Acutely, there was no evidence of cell escape and, during insulin secretion increase, biomass remained constant.

Apart from the studies conducted, there are ethical concerns relating to use of human embryos for generation of ESCs. iPSCs from somatic cells could be considered an appropriate source for large β-cell generation from a nonembryonic source [55]. Nevertheless, PSCs impose the possible threat of tumor formation and the need for differentiated cell purification is still an issue [56]. Overall, stem cells have proved to be a most promising cell source that could contribute to diabetes treatment; however, further studies must be performed to achieve sufficient β-cell production with large-scale cell differentiation, hoping that this can be applied at the clinical level, as well as the functional improvement of such systems via encapsulation.

Xenotransplantation of porcine islets has been an attractive approach owing to the close homology between porcine and human insulin and the similarities of the islets from the two species. Thus, porcine lines free of infectious porcine endogenous retrovirus have been identified. In the literature, the co-encapsulation of pig islets with adipose or bone marrow MSCs has been shown to improve implant oxygenation and neoangiogenesis, nevertheless it does not seem to ameliorate the long-term function of the BAP in a primate model post subcutaneous implantation [57]. More recently, Teotia and co-workers engineered a PSU/TPGS composite hollow fiber membrane (HFM) BAP and examined the xenotransplantation of encapsulated porcine islets and cell clusters derived from MSCs isolated from human umbilical cord Wharton's jelly. Normoglycemia was achieved for 30 days, and an immune response was avoided [58]. Further studies should be conducted to ensure the long-term behavior of this system.

Adult acinar and duct cells from the exorcine pancreas have been investigated for their reprogramming to β-cells [59,60]. Gastrointestinal cells can also be reprogrammed into insulin-producing β-cells through expression or deletion of some transcription factors, such as FOXO1 inhibition [61]. It is noteworthy that cells from the gastrointestinal epithelium have a propensity for conversion into insulin-secreting cells. However, the encapsulation of such cells *in vitro* or *in vivo* has not yet been tested [62]. Advances in cell sources for generations of insulin-producing cells are extensively described in a retrospective work [63]. Although there are encouraging studies in the literature, in terms of clinical application more research must be performed because reproduc-

ibility is an issue as no standard protocols have been designed [64]. Further study in the future could result in a new perspective in diabetes treatment with the aid of gene-editing technologies.

Oxygenation until angiogenesis

Pancreatic β-cells consume a large amount of oxygen during GSIS, because of the high demand of mitochondrial respiration [65]. Nutrient and oxygen ingress to encapsulated islets is facilitated by capillary ingrowth within the capsule [44]. During the early post-transplantation period, oxygen feed takes place only via diffusion until neovascularization of the graft. Therefore, islet hypoxia can occur, inhibiting insulin secretion and leading to cell apoptosis. Several strategies have been investigated to prevent hypoxia damage.

The use of certain compounds such as exendin-4 can attenuate the hypoxia outcome. For instance, it has been demonstrated before that exendin-4 protects β-cells from oxidative stress, enhances GSIS and improves the survival of porcine or mouse islet grafts in mice, whereas it stimulated insulin release in hypoxic islets [66]. Subsequently, the co-encapsulation of porcine islets with exenatide-loaded poly(lactide–co–glycolide) (PLGA) microspheres in alginate microcapsules was studied, accomplishing enhanced islet viability and GSIS function *in vitro* [67]. In addition, the vascular endothelial growth factor (VEGF) in encapsulation systems of polyethylene glycol maleimide (PEG–MAL) has been shown to augment vascularization [68]. Marchioli and co-workers constructed a scaffold consisting of an alginate-islet core, surrounded by a PCL-heparinized ring. Heparin enabled VEGF binding and increased angiogenesis was observed on and near the scaffold surface *in vitro* [69].

Recently, data from our group have shown that human pancreatic islets cultured and preserved under hypoxic conditions (1% O_2) overexpress insulin growth factor (IGF)-1 mRNA variants (unpublished data). Conceivably, this is an IGF-1-mediated survival mechanism protecting islets from hypoxia-induced apoptosis. Furthermore, using a rat pancreatic β-cell line (INS-1), we have documented that IGF-1 variants are downregulated when treated with proinflammatory cytokines [interleukin (IL)-1β, tumor necrosis factor (TNF)α and interferon (INF)-γ]. Therefore, we are proposing the investigation of the role of E peptides of the *igf-1* gene transcripts on INS-1 cells and their regulation by proinflammatory cytokines and hypoxia. We aim to clarify the role of IGF-1 isoforms and the synthetic E peptides on regulation of β-cells and their impact on β-cell mass and function.

Furthermore, hypoxia and cell necrosis can be avoided with the use of oxygen affinity carriers. Perfluorocarbons are widely used for oxygen boost because of their high oxygen solubility [46]. Lee *et al*. performed integration of perfluorodecalin (PFD) in alginate microcapsules and managed to ameliorate islet function and survival *in vitro* and *in vivo* by minimizing the hypoxic damage of mouse islets transplanted intraperitoneally in mice [70]. Significantly, HIF-1α, which can induce cell apoptosis, was not observed in the nuclei of the PFD-alginate capsules as opposed to nonencapsulated islets and alginate groups when exposed to hypoxia. The PFD-alginate capsules also demonstrated higher levels of GSIS under the hypoxic state. A hemoglobin, HEMOXCell®, has been verified to increase cell viability and decrease the hypoxia marker, when tested in BAP *in vitro* [71]. The fabrication of an oxygen-generating scaffold by mixing calcium peroxide (CaO_2) with poly(dimethylsiloxane) (PDMS) was performed and tested with porcine neonatal pancreatic cell clusters (NPCCs) *in vitro* where overall viability was improved. Encapsulated NPCCs showed lower caspase-3 and caspase-7 activity, hypoxic cell expression and reactive oxygen species (ROS) levels, and higher oxygen consumption rate (OCR) and GSIS [72]. Recently, manganese oxide (MnO) nanoparticles were examined for their cytoprotective and oxygen-generating properties, with potential use for cell therapies [73].

BetaO_2 manufactured the βAir device which provided an exogenous oxygen supply from a gas chamber to the islet compartment, achieving graft survival for 10 months. However, daily air refills were required [49]. An alternative route was introduced by Evron and co-workers, who developed a device where the oxygen supply is administered by the immobilized cyanobacterium *Synechococcus lividus,* through photosynthesis upon illumination [74]. Nevertheless, such a method would require an implantable battery and transcutaneous energy transmission for recharging [46]. Another strategy to alleviate hypoxia has been the implantation of a void encapsulation device in the host to allow pre-vascularization of the device and subsequent implantation of the islets [75]. Hence, in this case the choice of the device and the transplantation site should facilitate the device retrieval. The pre-vascularization of a subcutaneous transplantation site has also been suggested with a temporary medically approved vascular access catheter [14].

Immune response modulation

The transplantation of encapsulated islets can evoke the host immune response by releasing cytokines, chemokines, macrophages, free radicals and nitric oxide, which can, ultimately, result in graft failure [76]. In T1DM patients, proinflammatory M1 macrophages are activated leading to the secretion of chemokines, such as CXCL10 and CCL5, whereas the synthesis of cytokines such as TNFα and IL-1β, type I IFNs, and cell-surface co-stimulatory molecules, such as CD40 and CD80, is promoted. These components attack the sites of transplanted islets in diabetic patients provoking pancreatic β-cell necrosis [77–79]. In addition, fibrotic overgrowth around the microcapsule surface, because of foreign body response, hinders mass transfer and induces hypoxia.

In the literature, it is manifested that the incorporation of cytokines or chemokines in encapsulation systems can modulate the host immune response. Liu *et al*. investigated localized release of recombinant transforming growth factor-beta 1 (TGF-b1) from poly-lactide-co-glycolide (PLG) layered scaffolds. TGF-b1 delivery dampened the expression of TNFα, IL-12 and monocyte chemoattractant protein (MCP)-1 and the infiltration of leukocytes [80]. In addition, incorporated chemokine CXCL12 in alginate microcapsules ameliorated allo- and xeno-graft survival in a murine specimen, by regulatory T cell (T_{reg}) recruitment which promoted immunosuppression [81]. Previously, Graham *et al*. had shown that co-localization of T_{regs} with PLG-encapsulated scaffolds improved graft function, whereas host-derived T_{regs} replaced the transplanted ones over time [82]. Moreover, the modulation of the high mobility group Box 1 (HMGB1)-mediated inflammatory response has been investigated, in the literature, with a twofold improvement of islet survival rate in xenografts [83].

Pham and co-workers suggested the localized release of an immunosuppressant around the xenograft field. FK506-loaded PLGA–PEG nanoparticles, conjugated with 3,4-dihydroxyl-l-phenylalanine (DOPA), formed a multilayer nanocoating-camouflage

around pancreatic islets from rats which, subsequently, were xenotransplanted in diabetic mice and, ultimately, islet survival time was improved by 1.7-fold. FK506 seemed to hinder T cell activation and the production of cytotoxic cytokine IL-2 [84]. Similarly, another research group co-encapsulated lymphocytes and an anti-inflammatory drug, pentoxifylline, along with the pancreatic islets in alginate microcapsules coated with dextran-spermine. *In vitro* tests concluded that pentoxifylline inhibited IL-2 secretion by 64% [85].

ECM components are reported to support islet function in alginate microcapsules. LLacua *et al*. reported that the entrapment of collagen type VI in alginate at concentrations of 0.1 and 10 mg/ml for the microencapsulation of human pancreatic islets improved cell viability and oxygen consumption without influencing the GSIS from tests conducted *in vitro* [86]. The same group later incorporated specific ECM such as collagen type IV and laminins (RGD or PDSGR) in human islets enveloped in alginate microcapsules and observed enhanced islet survival with lower susceptibility to cytokine-mediated cytotoxicity. Indicatively, the encapsulated islets were exposed to IL-1β, IFN-γ and TNFα for 24 and 72 h, and impaired cell apoptosis was noted. Moreover, collagen-IV-RGD and collagen-IV-PDGRS reduced NO release from the encapsulated human islets, suggesting higher oxygen consumption [87].

Is has previously been stated that oxidative stress can contribute to an autoimmune response at an islet transplantation site [88]. It was also demonstrated that poly(*N*-vinylpyrrolidone)/tannic acid (PVPON/TA) nanothin coatings could significantly decrease *in vitro* chemokine synthesis and T cell migration, whereas (PVPON/TA)-encapsulated islets restored euglycemia after transplantation into diabetic mice [88]. Furthermore, the encapsulation material itself, as well as the shape, the porosity and the dimensions of the microcapsules, plays a dominant part in graft survival [45,89]. For instance, a foreign body response can cause pericapsular fibrosis in microencapsulation systems owing to material impurities as in the case of alginate [90]. However, clinical-grade commercially purified alginate has been reported to cause a foreign body response. Doloff *et al*. evaded macrophage-dependent biomaterial fibrosis by targeting colony-stimulating factor-1 receptor (CSF1R), utilizing the ATP-competitive inhibitor GW2580, when alginate, ceramic glass and PS spheres of 500 μm were tested in mice [91]. In general, functionalization of encapsulation materials can result in more-immunoprotective capsules [33,37–39,44].

Transition to clinical practice

Full clinical translation of the encapsulation strategy has not yet been accomplished; nevertheless, a substantial number of clinical trials of encapsulated islet transplantation with auspicious results have been conducted. Currently, five clinical trials have successfully been completed, and seven are active or at the recruitment stage (Table 3). Over 1500 procedures have already been performed worldwide in 40 international centers – 864 were allogeneic and 480 were autologous [92]. Encapsulation material and design, as well as implantation sites, are the key aspects of clinical trials. However, none of the published results has been associated with clear evidence of a fully functioning encapsulation strategy. Specifically, the attained normoglycemia was only accomplished in a few cases, at an insufficient level.

In 2007, Living Cell Technologies (LCT) performed a xenotransplantation trial in Russia to investigate the safety and effectiveness of Diabecell® in eight patients (LCT/DIA-07R, LCT/DIA-07R2). Owing to the promising initial results, LCT and Diatranz Otsuka proceeded in three Phase II trials that were performed in New Zealand and Argentina (NCT01736228, NCT01739829, NCT00940173). The objective was to determine the optimal dose and frequency transplantation of neonatal porcine islets encapsulated in alginate microcapsules. In all cases, reduction in exogenous insulin and hypoglycemic episodes were reported [93,94]. Moreover, the cohort that received the higher dose (i.e., 20 000 IQE/kg) showed better results [93].

In Belgium, a pilot study of a Phase II trial was coordinated by AZ-VUB to monitor the function of microencapsulated β-cells that were laparoscopically injected in the peritoneal cavity of a female patient who was on immune suppression (NCT01379729). The fibrotic tissue is surrounding the capsules [95]. Another clinical trial in Belgium was intended to assess the transplantation of a monolayer cellular device in the subcutaneous tissue, however it was finally withdrawn (NCT00790257).

In 2014, Uppsala University Hospital in collaboration with BetaO2 Technologies started an open-label, pilot investigation to assess the safety of βAir and investigate glycemic control in four patients (NCT02064309). One-to-two βAir devices, each containing 1800–4600 IEQ/kg of BW, were transplanted and monitored for 3–6 months [96]. However, although β-cells survived, their function was limited because only minute levels of circulating C-peptide were observed with no impact of metabolic control.

ViaCyte is in a Phase I/II trial in the USA and Canada to test whether VC-01™ can be safely and effectively implanted (NCT02239354). The total estimated enrolment is 56 participants divided into two cohorts implanted with: (i) two implants and (ii) four-to-six implants. Furthermore, the company took permission for a 1-year follow-up safety study in patients previously implanted with VC-01™ (NCT02939118). The purpose of this study is to report incidences of adverse events. An ongoing Phase II multicenter trial is currently being performed to investigate the safety and tolerability of VC-02™ – a new combination product for high-risk T1DM patients (NCT03162926). It is estimated that up to six devices of PEC-01™ per person will be implanted into 15 subjects. Currently, a multicenter Phase I/II trial is at the recruitment stage and is planned to occur into two stages in the USA and Canada (NCT03163511). First, two and six VC-02™ devices will be implanted into 15 subjects to report incidence of all adverse events. Consequently, the devices will be tested in the second cohort of 40 patients to measure the change in C-peptide.

An ongoing trial at the University of Miami is investigating allogeneic islet cell transplantation onto the omentum (NCT02213003). Islets are resuspended in autologous plasma and laparoscopically distributed on the omental surface. The omentum is a potential transplant site, because it has a dense vascularized surface and is easily accessible. Moreover, clinical-grade recombinant human thrombin is used for cell adherence. Initial results on a 43-year-old female patient were recently reported by Rodolfo Alejandro's group, indicating restored euglycemia and insulin independence [97]. Despite a functional decline in 12 months, the subject continued to have stable glycemic control. Although the trial occurred with constant immunosup-

TABLE 3

Completed and ongoing clinical trials of encapsulation systems

Identifier	Sponsor	Status	Interventions	Phase	Locations	First posted
NCT03513939	Sernova	Not yet recruiting	Combination product: Sernova Cell Pouch™	1,2	–	2018
NCT03163511	ViaCyte	Recruiting	Combination product: VC-02	1.2	University of California San Diego, Johns Hopkins University, University of Minnesota, Ohio State University, University of Alberta, University of British Columbia	2017
NCT03162926	ViaCyte	Active, not recruiting	Combination product: VC-02, (aka PEC-Direct)	1	University of Alberta	2017
NCT02939118	ViaCyte	Enrolling by invitation			–	2016
NCT02064309	Uppsala University Hospital	Active, not recruiting	Device: β-Air device for encapsulation of transplanted human islets	1,2	Uppsala University Hospital	2014
NCT02239354	ViaCyte	Active, not recruiting	Device: VC-01™ Combination product	1,2	University of California San Diego, University of Alberta	2014
NCT02213003	Rodolfo Alejandro, MD	Recruiting	Biological: islet transplantation, thrombin plasma gel	1,2	Diabetes Research Institute, University of Miami Miller School of Medicine	2014
NCT01736228	Living Cell Technologies	Completed	Device: DIABECELL	2	Hospital Interzonal General de Agudos Eva Perón San Martin	2012
NCT01739829	Diatranz Otsuka	Completed	Device: DIABECELL	1,2	Hospital Interzonal General de Agudos Eva Perón San Martin	2012
NCT00940173	Diatranz Otsuka	Completed	Device: DIABECELL	1,2	Centre for Clinical Research and Effective Practice	2009
ACTRN12609000192280	South Eastern Sydney Local Health District	Completed	Device: seaweed alginate microcapsule	1	South Eastern Sydney Local Health District	2009
LCT/DIA-07R, LCT/DIA-07R2	Living Cell Technologies	Completed	Device: DIABECELL	1,2	Sklifosovsky Institute Moscow	2004
ISRCTN43557935	University of Perugia	Completed		1	University of Perugia	2004

pression, success with the transplantation at this extrahepatic site could lead to superior functional and clinical outcomes for encapsulated islet transplantation.

Recently, a new clinical trial has begun (by Sernova) to test the safety, tolerability and efficacy of Cell Pouch™ (NCT03513939). The device will be loaded with >3000 IEQ/kg of BW and implanted under the skin in seven T1DM subjects with hypoglycemia unawareness and a history of severe hypoglycemic episodes. For proper vascularization of the device's chamber, immunosuppression will be initiated 3 weeks after the implantation.

Overcoming limitations of islet encapsulation

Encapsulation failure modes are either related to the process or occur *in vivo* after the implantation. During the isolation and encapsulation process, islets are adversely affected by processing stress owing to various conditions such as temperature, shear stress, hypoxia, among others. After implantation, the efficacy of the encapsulation approach can be limited owing to acute inflammation, hypoxia and foreign-body-mediated fibrosis. Chronic failure involves chronic hypoxia, antigen release caused by cell necrosis, immune activation and ramifications of mechanical failure.

With the goal being to overcome some of these challenges, various nano-, micro- and macro-encapsulating approaches have been developed. The main advantage of micro- and nano-encapsulation is the surface:volume ratio maximization and thus improvement of the diffusion of nutrients and insulin. However, ultimate control over the thickness and porosity of capsules to achieve enhanced diffusion is not fully accomplished. Conversely, macroencapsulation approaches are inferior to facilitating exchange and cell response resulting in delayed insulin secretion.

From a clinical perspective, encapsulation devices offer flexibility for transplantation site selection, ease of retrieval and reloading. Nevertheless, the small magnitude of devices decreases the load capacity entailing the need of manifold transplantation. Individually encapsulated islets have the advantage of uniform distribution throughout a cavity allowing the surface of the membrane to have contact with the host [98]. However, this approach is limited owing to difficulties in live imaging, tracking and retrieving.

In addition to the aforementioned challenges, a primary concern is the production through a cost-effective and labor-efficient method necessitated by the high requirements of a therapeutic dose. The fabrication process in most macroencapsulation devices

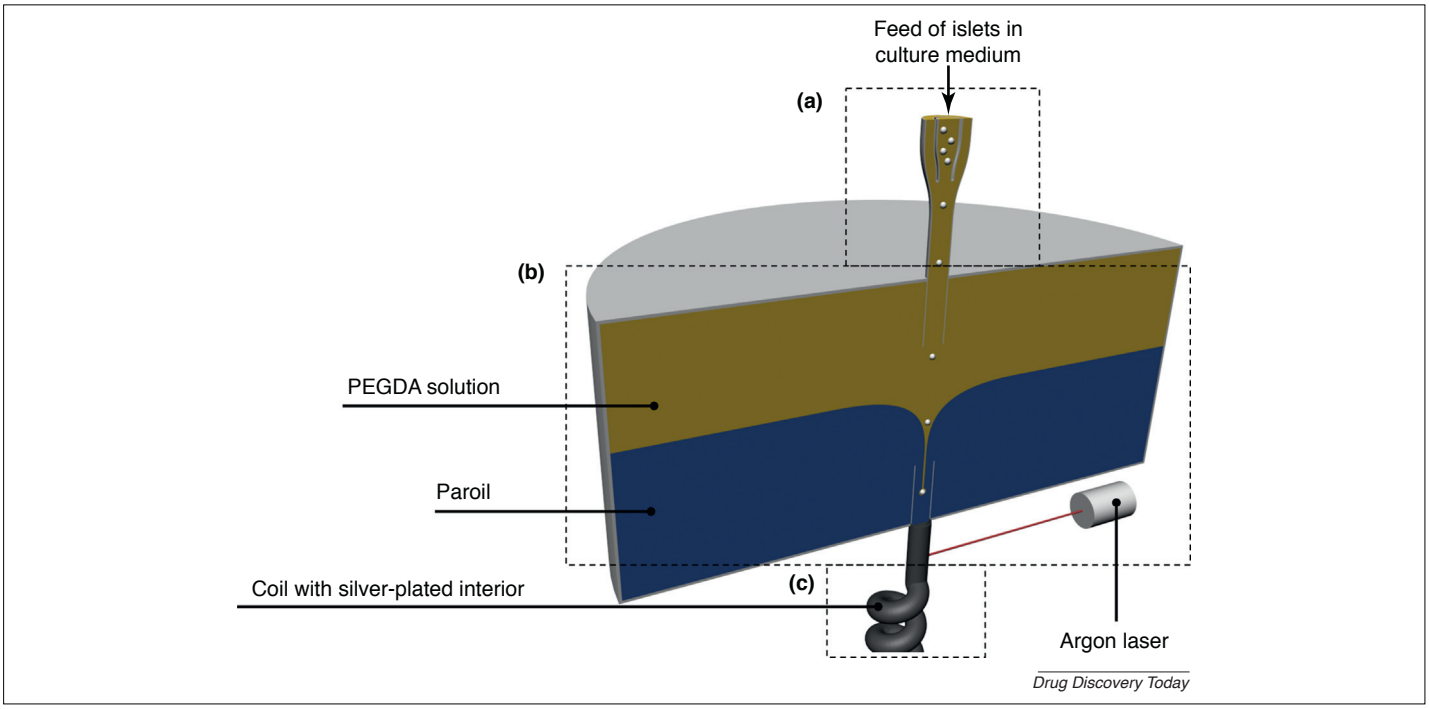

FIGURE 2

An islet microencapsulation apparatus paradigm, which consists of: **(a)** a feeder with hydrodynamic focusing, which aligns the islets in a single file to the withdrawal tube; **(b)** an encapsulation chamber, where the islets are coated by a solution of PEG-DA; and **(c)** a photopolymerization sector, where the polymer is crosslinked to form a conformal semipermeable membrane.

is simple but involves several steps and material design principles. 3D printing technology is already in use and can potentially accelerate the whole process. The most common technologies in microencapsulation are microfluidics, microelectromechanical systems and micromolding. It should be noted that batch approaches are more time-consuming and labor-intensive than continuous modes. Stochastic loading and islet separation, processing stress reduction, high yields and rates of individual encapsulation, and efficient polymerization are issues that must receive proper attention. A microencapsulation islet apparatus was recently designed aiming at efficiently encapsulating a high amount of islets within a reasonable period of time [99] (Fig. 2).

Concluding remarks and future outlook

Currently, basic and clinical research focuses on encapsulation material, transplantation site and methods to improve immune modulation and neovascularization. Many strategies have been proposed for the longevity and function of the graft by addressing hypoxia (refillable oxygen supply, artificial oxygen carriers), enhancing vascularization (growth factors, ECM proteins) and modulating immune response (scavengers). Despite these important advances, encapsulation technology continues to face significant obstacles for full clinical transition. Given the donor shortage, stem cells have the potential to be a replenishable source. With large-scale production of stem cells, the development and assessment of encapsulation strategies will be essentially accelerated. Nevertheless, further work is necessary to develop cells that have all the necessary characteristics in a sufficient number and without the risk of teratogenicity. Future success requires close collaboration between partners from different fields, to deeply understand the current limitations and to employ the advances summarized in this review.

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