



# Generation of Human Stem Cell-Derived Pancreatic Organoids (POs) for Regenerative Medicine

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## Abstract

Insulin-dependent diabetes mellitus or type 1 diabetes mellitus (T1DM) is an auto-immune condition characterized by the loss of pancreatic  $\beta$ -cells. The curative approach for highly selected patients is the pancreas or the pancreatic islet transplantation. Nevertheless, these options are limited by a growing shortage of donor organs and by the requirement of immunosuppression.

Xenotransplantation of porcine islets has been extensively investigated. Nevertheless, the strong xenoimmunity and the risk of transmission of porcine endogenous retroviruses, have limited their application in clinic. Generation of  $\beta$ -like cells from stem cells is one of the most promising strategies in regenerative medicine. Embryonic, and more recently,

adult stem cells are currently the most promising cell sources exploited to generate functional  $\beta$ -cells in vitro. A number of studies demonstrated that stem cells could generate functional pancreatic organoids (POs), able to restore normoglycemia when implanted in different preclinical diabetic models. Nevertheless, a gradual loss of function and cell death are commonly detected when POs are transplanted in immunocompetent animals. So far, the main issue to be solved is the post-transplanted islet loss, due to the host immune attack. To avoid this hurdle, nanotechnology has provided a number of polymers currently under investigation for islet micro and macro-encapsulation. These new approaches, besides conferring PO immune protection, are able to supply oxygen and nutrients and to preserve PO morphology and long-term viability.

Herein, we summarize the current knowledge on bioengineered POs and the stem cell differentiation platforms. We also discuss the in vitro strategies used to generate functional POs, and the protocols currently used to confer immune-protection against the host immune attack (micro- and macro-encapsulation). In addition, the most relevant ongoing clinical trials, and the most relevant hurdles met to move towards clinical application are revised.

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**Keywords**

Cell differentiation · Innovative therapies · Pancreatic organoids · Stem cells · Type 1 diabetes mellitus

**Abbreviations**

2D	Bi-dimensional	iPSCs	Inducible pluripotent cells
3D	Three-dimensional	KCl	Potassium chloride
A. ECM	Artificial ECM	LbL	Layer-by-layer
AHFBRs	Alginate-filled hollow fiber bioreactors	MGC	Methacrylated glycol chitosan
ALDHhi	High aldehyde dehydrogenase activity	MMP	Matrix metalloproteinase
BAPs	Bioartificial pancreas	MP	Microparticles
BCD	$\beta$ -cell-derived cells	mPEG	Methoxy polyethylene glycol
BM-MSC	Bone marrow MSC	MSC	Mesenchymal stem cells
CaO <sub>2</sub>	Calcium peroxide	NeuroD1	Neuronal Differentiation 1 protein
Cas9	DNA endonuclease Cas9	NICC	Neonatal islet-like cell clusters
CPO	Calcium peroxide	NICHE	Neovascularized implantable cell homing and encapsulation
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats	NK	Natural Killer T cells
Dex	Dexamethasone	NPCCs	Neonatal pancreatic cell-clusters
ECM	Extracellular matrix	NOD	Non-obese diabetic
ED	Eudragit	PANC-1	Human pancreatic ductal-cells
EPCs	Endothelial progenitor cells	PB	Probucol
FBM	Functional $\beta$ cell mass	PB-CDCA	PB-TCA alginate-microencapsulation
FICCs	Fetal porcine islet-like cell clusters	PCL/PVA	polyvinyl alcohol/polyvinyl alcohol
FTY720	Fingolimod	PDMS	Polydimethylsiloxane
GHRH	Growth hormone releasing hormone	PDX1	Pancreatic and duodenal homeobox 1
Glut-2	Glucose transporter 2	PEG	Poly(ethylene glycol)
HARV	High aspect ratio vessels	PEGCol	Poly(ethylene glycol) hydrogels containing collagen type I
Hep-PEG	Glycosaminoglycans and heparin enriched capsules	PEG-VS	Vinyl sulfone-terminated polyethylene glycol
hESCs	Human embryonic stem cells	PEOT/	Poly(ethylene oxide terephthalate)-
hHPCs	Human hepatic progenitor cells	PBT	poly(butylene terephthalate)
hiPSC	Human induced pluripotent stem cells	PERV	Porcine retrovirus
HLSC	Human liver stem cells	PES/PVP	Polyethersulfone/polyvinylpyrrolidone
HLSC	Adult human liver stem-like cells	PFTBA	Perfluorotributylamine
HMGB1	High mobility group box 1	PGA	Polyglycolides
huPI-MSc	Adult human pancreatic islet mesenchymal stromal cells	PLA	Poly(lactic acid)
IBMIR	Instant blood-mediated inflammatory reaction	PLGA	Poly(lactide-co-glycolide)
IL-2	Interleukine-2	PLLA/	Poly-L-lactic acid-polyvinyl alcohol
IPCs	Insulin producing cells	PVA	Poly-L-ornithine
		PLO	Poly-L-ornithine
		PMAA-Na	Sodium polymethacrylate
		POs	Pancreatic organoids
		PSCs	Pancreatic stem cells
		Ptf1a	Pancreas Associated Transcription Factor 1a
		PTFE	Polytetra-fluoroethylene

PTX	Pentoxifylline
RWV	Rotating wall vessels
SF	Silk fibroin macroporous
SIPN	Semi-interpenetrating polymer network
SP	Saccharide-peptide
SPO	Sodium percarbonate
starPEG	Star-shaped polyethylene glycol
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TCA	Taurocholic acid (bile acid)
Th	T-helper
TRAFFIC	Thread-reinforced alginate fiber for islets encapsulation
TNF- $\alpha$	Tumor necrosis factor-alpha
VEGF	Vascular endothelial growth factor
VEGFR2	Vascular endothelial growth factor receptor 2

## 1 Introduction

Type 1 diabetes mellitus (T1DM) develops when the endocrine pancreas fails to produce proper insulin concentrations to maintain the glucose homeostasis. The current approach to treat T1DM patients consists in the administration of exogenous insulin to supply the deficiency of pancreas secretion. This implies that pancreas transplantation would represent the finest therapeutic approach in these patients.

The protocols applied to isolate and purificate adult human pancreatic islets have been standardized and considerably improved over the last years (Brennan et al. 2016). Advances in peritransplant anti-inflammatory strategies, immunomodulation, and immunosuppression, have also contributed to the improvement of current clinical results (Ricordi et al. 2016). Nevertheless, only ~50% of recipients remain insulin-independent 5 years after pancreas transplantation. This mainly depends on the related inflammatory reaction, hypoxia and hypoxia-reoxygenation injury (Pepper et al. 2018).

Transplant of pancreatic islets is currently considered an approach to be used in highly selected

patients with severe hypoglycemia or instable T1DM (Liu et al. 2015). For decades, the clinical application of islet transplantation has been limited by the inadequacy of the immunosuppressive therapy and the number of donor tissues (Liu et al. 2015). Once transplanted, only nearby 60% of the T1DM patients become insulin independent and maintain the glycemic control (Liu et al. 2015). In addition, most of these patients gradually lost insulin independence 2 years after transplantation (Shapiro et al. 2006). Beneficial long-term outcomes consisting in a long-term insulin independence ( $\geq 5$  years) depends on an efficient immunosuppressive therapy able to improve islet mass engraftment and prevent recurrent autoimmunity (Bellin et al. 2012). However, long-term immunosuppressive therapy is associated with a significant morbidity, including the increased risk of cancer and infections (Shapiro et al. 2017). Moreover, as extensively demonstrated, nutrients cannot effectively reach the core of large-size transplanted islets resulting in cell death and islet loss. Such diffusional problems could be solved by breaking up islets into single islet cells. However, dissociated cells are less efficient than intact islets. Based in this concept, Li et al. (2017) established a novel islet engineering approach by encapsulating dissociated cells from human islets to generate newly formed islet-like organoids, similar in size and gene expression profile (Isl-1, Gcg, and insulin-1) to native islets. Furthermore, by limiting the diameter of these engineered islet cell clusters, to a maximum of 100  $\mu\text{m}$ , cell viability and insulin secretion were improved (Li et al. 2017). Nevertheless, this approach faced the same problem as pancreas and islet transplantation, the shortage of donor organs (Matsumoto 2010).

Additionally, the lost of post-transplanted islets, due to the adverse immune and non-immune reactions (Croon et al. 2003), is associated with the failure of long-term insulin independence after intrahepatic islet transplantation (Bruni et al. 2014). This implies that to increase the number of transplantable T1DM patients, it is mandatory to obtain reliable and standardized sources of human transplantable islets, and avoid immunosuppression. To cover these needs, several efforts have been devoted to develop new potential

strategies to produce functional pancreatic organoids (POs) (Zhou and Melton 2018). However, similar to pancreas or islet transplantation, transplanted POs activate the host immune response (Szot et al. 2015) which targets POs and leads to their loss of function (Szot et al. 2015).

Xenotransplantation using porcine islets, has been considered as an alternative source of human islets, and extensively investigated (Cardona et al. 2006; Hering et al. 2006). The easy and less expensive isolation procedure of fetal porcine islet-like cell clusters (FICCs) and neonatal islet-like cell clusters (NICCs) represents the main advantage at using xenotransplantation instead of allogenic/heterogenic allografts (Liu et al. 2017). Significant improvement in glycaemic control, has been provided by xenotransplantation (Liu et al. 2017). However, after transplantation, islet xenografts gradually lose efficacy (Safley et al. 2018a). Furthermore, the strong xenoimmunity and the risk of porcine endogenous retroviruses transmission, have limited their application in clinic (Liu et al. 2017; Van Der Laan et al. 2000; Samy et al. 2014).

In the last decades the research has been focused on the identification of alternative cell sources from which functional insulin-secreting cells could be generated (Matsumoto et al. 2016). Cell lines are currently considered as potential sources able to generate new and mature insulin-secreting cells (Ravassard et al. 2011; Boss et al. 2017). For a long time, the introduction of the insulin gene along with components of the stimulus–secretion coupling pathways have been applied to induce the commitment of immature cells to insulin-producing cells. However, these cells failed to effectively store and release fully processed insulin (Ravassard et al. 2011; Clark et al. 1997; Scharfmann et al. 2014; Xie et al. 2016). Stem cells, isolated from embryonic and more recently, from adult tissues, are currently the most promising approaches (Balboa et al. 2018). Despite the great efforts and the promising results, so far, cell products covering all the morphological and functional profiles

recapitulating the mature pancreatic islets are still missing.

Pancreatic islets are three-dimensional structures (3D) composed by different endocrine cells. The cellular interactions resulting from this 3D conformation play an important role in the modulation of hormone secretion (Navarro-tableros et al. 2018; Kim et al. 2016a). Assembly of stem cells in 3D structures facilitates cellular interactions and promotes morphogenesis and tissue organization mimicking native pancreatic islets (Navarro-tableros et al. 2018; Kim et al. 2016a). Both natural and artificial materials can be used to create 3D synthetic organic and inorganic porous scaffolds (Hinderer et al. 2016). Nanotechnology have provided new opportunities to design distinctive materials with specific physicochemical properties (Liu et al. 2018). Thus, generation of POs similar, in terms of morphology and function to native pancreatic islets, not only may enhance long-term transplant viability in clinical settings, but could also be applied for drug screening purposes (Scavuzzo et al. 2018). Hydrogels are extensively used for drug delivery and tissue regeneration due to their similarity in terms of structure and biocompatibility to the native extracellular matrix (ECM) (Dimatteo et al. 2018; Steffens et al. 2018). Biomaterials are classified in three major groups: (i) naturally-derived materials; (ii) synthetic polymers; and (iii) decellularized organ- or tissue-derived scaffolds.

The size of organoids should be also considered to generate encapsulated POs. In general, it is widely accepted that smaller capsules provide better  $\beta$ -cell oxygenation (Buchwald et al. 2018). However, concerns related to their removal still remains if compared to macroencapsulation devices (Scharp and Marchetti 2014). Preliminary studies have suggested that islet cell encapsulation within meter-long microfibers might overcome this issue (Dolgin 2016). In this review, all 3D isolated structures (islets) or artificially generated, either encapsulated or not, and independently of their origin (human and other species) will be referred as POs.

In summary, key challenges for human translation still include: (1) generation of biocompatible materials, (2) generation of 3D POs with functional insulin secreting cells, (3) improvement of PO survival, (4) protection of POs from the immune attack, and (5) the availability of nutrient supply (Tomei et al. 2015). Therefore, the ideal encapsulation method, should allow long-term survival and functional capability of implanted POs (Ryan et al. 2017). Encapsulated islets or newly generated POs are typically delivered in extrahepatic sites such as intraperitoneally, subcutaneously or into the omentum (Berman et al. 2016). However, the host immune response and the activation of the fibrotic process occurring after transplantation, still remain a challenge (Safley et al. 2018a).

In this review, the new strategies to generate *in vitro* POs (stem cells-based, gene editing and alternative 3D culture methods), immunoisolation approaches, such as macro- and micro-encapsulation, and polymers used for these purposes will be discussed. Moreover, the ongoing clinical trials, and the hurdles to move towards their clinical application, will be also overviewed.

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## 2 The Relevance of 3D Structure

Islet architecture, including arrangement and interaction between endocrine cells is relevant for their precise function (Orci et al. 1975; Kitasato et al. 1996; Kanno et al. 2002). More importantly, the homologous and heterologous intercellular contacts are relevant to provide a finest regulation of insulin secretion (Wojtuszczyński et al. 2008). Furthermore, the paracrine and autocrine interaction between  $\alpha$ - and  $\beta$ -cells, contributes to the firm dynamic of hormone secretion, on which depends the effective control of glucose homeostasis (Jo et al. 2009). Additionally, gap-junction-mediated cell-cell interactions protect pancreatic cells against apoptosis (Klee et al. 2011), and correlate with a correct developmental acquisition of a mature insulin secretory profile (Santos-Silva et al. 2012). Therefore, the 3D architecture and the

intercellular regulatory mechanisms involving ions (electrical coupling) and hormones, particularly involved in the cross-talk among  $\alpha$ -,  $\delta$ -, and  $\beta$ -cells within the islets, are crucial to ensure a correct response to paracrine signals (Rorsman and Ashcroft 2018). Modification or loss of these properties results in abnormal glucose control (Rutter and Hodson 2015; Kilimnik et al. 2011). Upon islet isolation, insulin-producing cells undergo multiple cell death processes including apoptosis, anoikis, and necrosis, which have been attributed to the loss of critical interactions between cells, ECM and the vasculature (Paraskevas et al. 2000; Irving-Rodgers et al. 2014; De Vos et al. 2016). Modification of the islet architecture also occurs during isolation and culture of cadaveric human islets, prior to transplantation (Lavallard et al. 2016) or after infusion into the portal vein (Henriksen et al. 2012). This results in the failure to obtain a fine glycemic metabolic control (Morini et al. 2006). Therefore, the preservation of 3D cellular architecture of human islets or POs, derived from different cell sources, have a functional relevance for islet engraftment (Navarro-tableros et al. 2018). Improved protocols/devices for PO *in vitro* culture may thus offer crucial indications for appropriate growth and differentiation of stem/progenitor cells (S/PCs), allowing safeguarding of specific characteristics, such as multi-lineage differentiation capabilities and paracrine activity.

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## 3 Microencapsulation

Native pancreatic islets are heterogeneous structures formed by almost 5 different endocrine cells:  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ,  $\epsilon$ , and pancreatic polypeptide (PP) cells which by interacting in a complex and synchronized manner allow a fine autocrine and paracrine regulation. Generation of POs, reminiscent of native pancreatic islets in structure and function, should be pursued to provide a more efficient treatment. Indeed, generation of pancreatic endoderm and endocrine cells in 3D micro-environment has been extensively documented. Wang et al. (2017) have generated human embryonic stem cells (hESCs)-derived POs expressing

high levels of  $\beta$ -cell markers such as Pdx1, Ngn3, Insulin, MafA, and the Glucose transporter 2 (Glut-2). More importantly, they demonstrated that these POs contained pancreatic  $\alpha$ ,  $\beta$ ,  $\delta$ , and PP producing cells. Insulin biosynthesis was confirmed by the high C-peptide expression and by the presence of insulin-secretory granules. The improvement of insulin secretion response to high glucose concentration has finally demonstrated their functional maturation (Wang et al. 2017).

### 3.1 Nanotopographies as Drivers of Cell Differentiation

A number of studies strongly provided evidences that engraftment, proliferation and differentiation of stem cells into a specialized phenotype are modulated by their structural organization (Guilak et al. 2009). The interactions between extracellular binding sites and cytoskeletal elements strongly affect *in vitro* cell morphology, adhesion, and consequently gene expression (Bettinger et al. 2009).

Nanotopographies are involved in stem cell adhesion, and regulate their functions by mechanotransduction signals through the cell membrane (Yim et al. 2010). On the other hand, nanotopographies inducing lower cell adhesion cues, allow cells to form POs by promoting cell–cell interaction (Shen et al. 2015). Evidences have been also provided that differentiating hESCs and iPSCs are able to perceive nanotopographical signals, and efficiently differentiate into pancreatic cells (Kim et al. 2016b). It has been also described that compared to traditional 2D cultures, human iPSCs (hiPSCs) cultured in nanofibrous scaffolds and driven to differentiate into POs are formed by insulin-producing cells, and exhibit a morphological and functional profile similar to mature pancreatic  $\beta$ -cells (Nassiri Mansour et al. 2018).

The establishment and preservation of a functional  $\beta$ -cell mass (FBM) are replacement. FBM is dogged by a direct sensing of its key components, i.e. the number and function of  $\beta$ -cells (Pipeleers et al. 2008). Contrasting results

have been obtained by De Mesmaeker et al. (2018). They demonstrated that a sustained FBM could be obtained using immature proliferative porcine  $\beta$ -cells, but not human adult islet cells. These observations suggest that differentiation depends not only on the employed methodology, but also on the cell of origin.

Thereby, cell-based therapy requires simple and safe encapsulation methods to produce POs of uniform size and shape. Indeed, encapsulation promotes not only a quick cell aggregation of mesenchymal stem cells (MSC) in dense cell clusters, but also increases the expression of insulin and Pdx-1 mRNA (Barati et al. 2018). These concepts are currently exploited and widely applied in biomedical areas (Lee et al. 2011).

Encapsulation has been also pursued to provide immuno-isolation biocompatibility, optimized nutrient diffusion and insulin release by POs (Orive et al. 2015). In line with these needs, a number of new polymers have been generated (Gálvez-Martín et al. 2017). Besides the characteristics and configuration of microcapsules, the nature of the cells should be also considered. A number of different cell sources has been investigated for cell therapy including stem cells (autologous and allogenic), mature somatic cells, modified human cells, xenogenic cells and others (Kang et al. 2014). Alginate-based microcapsules have been reported not only to enable the proliferation and differentiation of hESCs into definitive endoderm-derived cells, but also to enhance their viability and proliferation, and to promote their aggregation into POs (Chayosumrit et al. 2010).

Independently of the origin, single cell within microcapsules, tend to spontaneously self-aggregate to form POs (Chayosumrit et al. 2010). These POs tend to mimic the structure and the features of the original *in vivo*-like cytoarchitectures (Wang et al. 2006; Zhang et al. 2006). Moreover, it is important to keep in mind that spheroid size also influences microcapsule efficacy (Perignon et al. 2015). POs with small diameters and with a low cell content, could not provide suitable conditions for cell aggregation due to inadequate number of adhering cells,

while large POs can undergo oxygen depletion with consequent hypoxia (Huang et al. 2012).

One of the most commonly used islet microencapsulation biomaterial is the alginate. Microcapsules have an ideal surface/volume ratio, which allows a better exchange of nutrients, insulin and glucose. Furthermore, islets could individually be included in a single capsule (Ryan et al. 2017; Desai and Shea 2017). Alginate is an anionic polysaccharide composed by unbranched polymers of 1,4-linked  $\beta$ -D-mannuronic and  $\alpha$ -L-guluronic acid residues which forms a gel in the presence of multivalent cations such as  $\text{Ca}^{2+}$  or  $\text{Ba}^{2+}$  and provides a 3D biomimetic environment to the cells that resembles the *in vivo* conditions (Datar et al. 2015). An important issue of first alginate products relied on their chemical instability during long-term implantation that hindered feasibility of cell therapy. It has been reported that alginate- $\text{Ca}^{2+}$  hydrogels tend to degrade, thus resulting in the contact of encapsulated structures with the host's immune system (Scharp and Marchetti 2014). Clinical and pre-clinical studies have noted that reducing the volume of encapsulated materials and the corresponding diffusional restrictions are critical for the engraftment (Scharp and Marchetti 2014). Experts in biomaterials have improved the stability of alginate hydrogels by modifying their biomechanical properties by a process called "click" crosslinking (Breger et al. 2015). These modifications confer to the capsules a superior stability, but a higher permeability to small size "diffusates". The alginate matrix can stabilize the cell cluster size and can allow a more homogeneous cell morphology, leading to a long-term culture of these clusters compared to non-encapsulated ones (Formo et al. 2015). Moreover, alginate-encapsulated human stem cell-derived POs could be effectively protected from the immune reaction when intraperitoneally implanted in mice (Vegas et al. 2016). In fact, micro-encapsulation is the most investigated approach (Zimmermann et al. 2007), and a number of pre-clinical studies in non-human primate models (Sun et al. 1996; Elliott et al. 2005), and human trials (Soon-Shiong et al. 1994; Calafiore et al. 2006; Tuch

et al. 2009; Jacobs-Tulleneers-Thevissen et al. 2013) based on this technology are ongoing.

Polymers applied to produce microcapsules should also have chemo-mechanical stability, and an easy to handle and appropriate pore size to allow a bi-directional diffusion of molecules in the semipermeable membrane (Kang et al. 2014). Currently, microcapsules have been obtained in many different forms, sizes, compositions, and with different permeability (De Vos et al. 2014). Both synthetic and natural polymers have been used for encapsulation purposes. Nevertheless, only alginate has been largely studied and it is currently certified as safe for human application (De Vos et al. 2014). As other polymers, alginate tends to be largely contaminated by original and additional contaminants such as polyphenols, endotoxins, and proteins introduced during the industrial extraction processes (Vos et al. 2006). In fact, a number of medicated alginates has been generated to solve this issue.

In general, it is accepted that smaller capsules (0.5 mm) better engraft functional POs (Buchwald et al. 2018). However, recent investigations suggested that the biocompatibility of alginate-encapsulated POs can be significantly enhanced by the use of larger capsules. In particular, it has been reported that larger capsules (1–1.5 mm) could generate a reduced immune reaction and fibrosis and avoid altered pattern of glucose-induced insulin secretion than smaller spheres (Veisoh et al. 2015). In contrast, other studies indicated that delayed and blunted responses to glucose and KCl depolarization, are present in larger capsules. As a consequence, larger capsules provide a reduced insulin response and a sustained and slow release of insulin in response to glucose (Buchwald et al. 2018).

A number of new microcapsules enriched with different combination of factors, has been developed. Recently, new capsules generated by a combination of antihyperlipidemic drug (probuco; PB), bile acid (taurocholic acid; TCA) and alginate-microencapsulation (PB-Alginate) called PB-CDCA capsules have been described. They are able to improve PO function and to reduce cell apoptosis driven by the hyperglycemic setting (Mooranian et al. 2016). Additional effects include

improved viability under the hyperglycemic environment, increased insulin production, and reduced TNF- $\alpha$  release by pancreatic  $\beta$ -cells (Mooranian et al. 2018). Furthermore, the PB-CDCA capsules, offer the mechanical stability, the buoyancy, the PB release, the thermal stability, and contain antioxidants (Mooranian et al. 2018). Similar results were obtained when capsules were enriched with absorption-enhancer chenodeoxycholic acid and Eudragit (ED) polymers (Mooranian et al. 2018) or ECM proteins (Mooranian et al. 2018). Moreover, it has been demonstrated that the addition of ECM proteins or trophic factors to alginate-encapsulated POs stabilizes the cluster size, cell morphology, and improves the oxygen consumption rate and PO survival in long-term culture (Formo et al. 2015). A newly ECM-based encapsulation system, named meter-long core shell alginate-hydrogel microfibers, has been shown to allow the formation of a core of pancreatic islet cells surrounded by ECM proteins and is considered a promising approach which can facilitate PO implantation and removing (Onoe et al. 2013).

The microencapsulated POs composed by pig islets seeded in human decellularized collagen matrix has been recently implanted in non-human primates (Dufrane et al. 2010). Long-term PO survival and insulin release were associated with improved glycemic control for 6 months, in the absence of immunosuppressive medications (Dufrane et al. 2010). However, the risk of porcine retrovirus (PERV) transfer, even if reduced, was still detected (Dufrane et al. 2010), possibly due to the damage of the alginate barrier (Crossan et al. 2018). Thus, additional quality control is mandatory before moving toward their clinical application.

By investigating alginate-encapsulated adult porcine islet transplants, it has become evident that despite long-term glucose normalization, a massive fibrosis occurred in harvested capsules (Vaithilingam et al. 2011). Nevertheless, improvement in avoiding fibrosis was obtained by using microcapsules enriched in photocrosslinked methacrylated glycol chitosan (MGC) (Hillberg et al. 2015) or Rampamycin-PEG (Park et al. 2017). Although relevant data have been obtained long-term and large

preclinical studies are still required (Park et al. 2017).

### 3.2 Immunomodulation

Even if the donor and organ managements, the surgical procedure, and the recipient management have been improved, the immune rejection still remains the most relevant issue to be solved (Schuetz et al. 2018). In order to safeguard graft survival, robust immunosuppressive treatments are required, some of which are toxic for  $\beta$ -cells (Nir et al. 2007). It has been demonstrated that the addition of multilayers composed by polymers, such as poly-L-ornithine (PLO) or activated methoxy polyethylene glycol (mPEG) to the surface of alginate microcapsules could allow immune protection and decrease interleukine-2 (IL-2) secretion (Nabavimanesh et al. 2015).

As above discussed, encapsulation is an effective approach to protect transplanted cells from rejection by the host immune response by using a semi-permeable artificial membrane. Microencapsulation has the advantage to allow implantation of allogeneic and xenogeneic cells, and free bidirectional diffusion of nutrients, oxygen, and other molecules like insulin. Moreover, it is also able to prevent the host immune response (Steele et al. 2014). Thus, efficient encapsulation approaches should be pursued to reduce or abolish the requirement of pharmacological immunosuppression. For example, double-layer alginate/dextran-spermine microcapsules combined with pentoxifylline (PTX) have been shown to be effective in preventing the immune attack over standard alginate-based encapsulation (Azadi et al. 2016). PTX could be easily released from the porous microcapsules, however, extended destruction of the membrane capsules has been documented (Azadi et al. 2016).

Alternative approaches aimed to improve immune-protection consist on the blockage of one of the most expressed proteins on the islets: the high mobility group box 1 (HMGB1) which is known to induce inflammation (Wang et al. 2016). Therefore, the regulation of HMGB1-mediated inflammation and particularly of the HMGB1 A



box (Itoh et al. 2011) is actually considered a new target for immuno-protection (Sama et al. 2004). In fact, recently, an anti-HMGB1 receptor-enriched encapsulation approach has been developed (Jo et al. 2015). Jo et al. (2015) have demonstrated that HMGB1 A box offers a protective effect on islet transplantation by decreasing the amount of TNF- $\alpha$  secreted by macrophages. It was also demonstrated that the HMGB-enriched-encapsulated method strongly improves PO survival rate after intraperitoneally xenotransplants into diabetic mice (Jo et al. 2015).

### 3.3 Encapsulated Xenografts

The possibility to use encapsulated porcine-islets to treat diabetes has been also evaluated. Promising results indicate that encapsulated porcine islets are more efficient than non-encapsulated porcine islets in restoring normoglycemia in diabetic monkeys (Sun et al. 1996). Co-encapsulation of pancreatic islets with MSC further improved vascularization and oxygenation of the PO graft (Vériter et al. 2014). However, despite a glycemic control was detected up to 32 weeks, not substantial improvement in the xenograft function was observed (Vériter et al. 2014).

A first phase I/IIa clinical trial aimed to test the microbiological safety of porcine islet xenotransplantation has been opened by recruiting nonimmunosuppressed subjects with unstable T1DM (Wynyard et al. 2014) transplanted with microencapsulated neonatal porcine islets (Wynyard et al. 2014). A reduction in unaware hypoglycemia events, the HbA1c levels, and a decrease of daily insulin requirement have been reported (Matsumoto et al. 2014). Pig-islet-based PO biosafety was further demonstrated in a phase IIa efficacy trial in humans (Morozov et al. 2017). Interestingly, plasma porcine C-peptide level has been applied to demonstrate the function of alginate-encapsulated porcine  $\beta$ -cells implanted in large and small animals (Montanucci et al. 2013; Chen et al. 2015). However, insight on the relationship between the initial  $\beta$ -cell dose, the viability of the implanted  $\beta$ -cells and their

function in term of metabolic control within time, are still missing (Dufrane et al. 2006; Foster et al. 2007).

It is important to remind that notwithstanding the relevant progress in xenotransplantation, significant limitations to clinical application persist:

1. No clinical applicable immunosuppressive protocols for preventing xenograft rejection are available (Samy et al. 2014).
2. High number of designated pathogen-free donor pancreata are required to manufacture neonatal porcine (Thompson et al. 2011) and adult pig islets for each patient (Rogers et al. 2011).
3. Manufacturing of NICC is still challenging and costly (Korbitt et al. 1996).

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## 4 Macroencapsulation

Human pancreatic islets are usually transplanted in the intrahepatic site, via the portal vein. However, this environment directly exposes the islets to the blood flow, thus eliciting the “instant blood-mediated inflammatory reaction” (IBMIR) and leads to islet damage and loss (Moberg et al. 2002; Nilsson et al. 2011). In addition, the concomitant reduced oxygen tension and the high intrahepatic concentrations of immunosuppressants further interfere with survival and engraftment of islets (Carlsson et al. 2001; Olsson et al. 2011; Desai et al. 2003). Unlike liver, the subcutaneous space has been proposed as an ideal implantation site, since it possesses a high grade of vascularization and can offer mechanical protections to the implanted islets (Pepper et al. 2015).

Even if transplantation of pancreatic islets is currently considered a promising therapeutically approach to cure diabetes, islet availability is limited by the number of donors and a large number of transplanted patients still requires insulin therapy to obtain the glycemic control (Shapiro et al. 2006). Additionally, due to the high oxygen demand, a highly vascularization is necessary to allow the long-term survival and function of transplanted islets. Furthermore,

accessible and minimally invasive sites are fundamental to allow implantation, replenishment and graft retrieval (Pagliuca and Melton 2013).

Recent advances in regenerative medicine and tissue engineering have preferred new strategies for the generation of 3D scaffolds. Thus, a number of biodegradable and biocompatible synthetic polymers have been used to produce nanofibers with the aim to improve maturation and function of the newly generated POs (De Vos et al. 2014). These materials rapidly undergo adaptation to the human body and do not stimulate the immune system (Ellis et al. 2017). These platforms provide a 3D environment that improves stem cell differentiation into  $\beta$ -like cells (Nadri et al. 2017; Abazari et al. 2018). It appears that these 3D nanofiber scaffolds are able to support an efficient cell-ECM interaction and cell-cell contact, by mimicking the *in vivo* condition (Mahboudi et al. 2018).

Macroencapsulation is an old technology also applied in diabetes research to provide a better survival of POs after transplantation (Desai and Shea 2017). Macroencapsulation devices are heterogeneous in geometry and in materials (Song and Roy 2016). A number of studies has shown the ability of pancreatic progenitor cells or endocrine cells to persist and function within subcutaneously implanted devices (Agulnick et al. 2015).

#### 4.1 Differentiation of Macroencapsulated Cells

Compared to 2D cultures, PCL/PVA 3D scaffolds could efficiently improve differentiation of iPSCs into  $\beta$ -like cells by inducing the expression of endocrine markers such as Glucagon, Insulin, Pdx1, Ngn3 and Glut-2 genes (Enderami et al. 2017). Differentiation properties of Poly(lactide-co-glycolide) (PLGA)-based microporous scaffolds have been also documented (Blomeier et al. 2006; Salvay et al. 2008; Mao et al. 2009). Poly(ethylene glycol) hydrogels containing collagen type I (PEGCol) have been also reported to promote aggregation and differentiation of hESC-derived progenitors in glucose-sensitive POs, also displaying enhancement of long-term viability

and morphology preservation (Mason et al. 2009; Amer et al. 2015). One of the most promising materials currently used for the generation of encapsulated POs is the poly-L-lactic acid-polyvinyl alcohol (PLLA/PVA) polymer, which provides a better microenvironment than 2D cultures (Mobarra et al. 2018). It has been reported that PLLA/PVA polymer is able to promote differentiation of iPSCs into insulin producing cells (iPSCs-IPCs) by inducing the expression of pancreatic-specific transcription factors such as Pdx1, insulin, glucagon and Ngn3 (Mobarra et al. 2018).

#### 4.2 Macroencapsulation and Immunoprotection

Bioscaffolds used as cell or drug carriers are usually constituted of non-degradable or degradable biomaterials. Current methodologies use ECM and ECM-like materials, or ECM-synthetic polymer hybrids (Hinderer et al. 2016). Hydrogels have been exploited for regenerative applications due to their biocompatibility and similarity in structure to the native extracellular matrix (Dimatteo et al. 2018). Several aliphatic polyesters, such as polymers of lactic acid [polylactides (PLA)], glycolic acid [polyglycolides (PGA)], and their copolymers [polylactoglycolides (PLGA)] have been extensively selected as biocompatible and bioresorbable matrices to reconstitute soft and hard synthetic engineered tissues (Mironov et al. 2017).

Polycaprolactone and polyvinyl alcohol (PCL/PVA)-based scaffolds display biological characteristics which improve differentiation of pancreatic  $\beta$ -like cells and organization into islet-like structures. PCL is a hydrophobic polyester which is biocompatible and biodegradable and has high mechanical stability. It is therefore considered a good candidate to produce 3D culture (Zarekhalili et al. 2017). Recent studies have demonstrated that the low-immunogenic polyethylene glycol (PEG)-based hydrogels used for islet transplantation have the ability to support islet engraftment and function (Jeong et al. 2013; Rengifo et al. 2014) and allow long-term function and restoration of normoglycemia in diabetic mice

(Rios et al. 2016). Some studies demonstrated that using polymer films and electrospun meshes made with poly(ethylene oxide terephthalate)-poly(butylene terephthalate) (PEOT/PBT) blocks copolymer, provides a protective environment to preserve islet morphology by preventing their aggregation in implanted islets (Buitinga et al. 2013). However, even if these scaffolds can allow a higher nutrient diffusion (glucose flux), and maintain the insulin and glucagon expression, a decrease of  $\beta$ -cell density in the islet core was observed (Buitinga et al. 2013). Further studies demonstrated that cell death, resulting from non homogeneous vascularization within the core and the outer shell of the scaffold-seeded islets (Buitinga et al. 2013), could be avoided by optimizing the pore size of the PEOT/PBT-based scaffolds (Buitinga et al. 2017).

In addition, it has been shown that microporous scaffolds, made with non-degradable polyethylene glycol (PEG)-based hydrogels, protect islets from the host immune response and show comparable results to the initial engraftment and function of non-encapsulated islets. Furthermore, unlike encapsulated islets which lose vascular connections to the host tissue, the microporous scaffolds allow islet revascularization after transplantation (Rios et al. 2018).

Recent progresses in nanotechnology and materials used for encapsulation, as well as in immunomodulatory strategies have significantly improved vascularization. Meanwhile, differentiation strategies of ESC and iPSC into islet-like structures have achieved an upscalable production for potential clinical applications. However, these cells can potentially drive tumour development (Päth et al. 2019). New strategies based on micro- and nano-materials (i.e., PEG, PLGA, chitosan, liposomes and silica) alone or enriched with trophic factors, have been recently developed to achieve a better stability of the capsules and, more importantly, to avoid loss of  $\beta$ -cell mass and PO function (Hinderer et al. 2016). Bioengineers are focusing on the development of new synthetic materials able to maintain tissue- and organ-specific differentiation and morphogenesis. Indeed, the newly generated bio-products have important advantages such as (1) a great surface

area for oxygenation and nutrient/catabolite transport, (2) a porous structure allowing infiltration of cells and blood vessels, (3) a satisfactory mechanical strength supporting cell attachment, (4) could be easily implanted, and (5) eventually, undergo degradation over time (Lutolf and Hubbell 2005).

To promote vascularization of transplantable devices housing pancreatic islets is the use of TheraCyte™ system. TheraCyte™ device is made by a bilayered polytetra-fluoroethylene (PTFE) where either free or microencapsulated islets are placed in the membrane to obtain a planar, bilaminar membranous pouch (Sörenby et al. 2008). A first clinical study has demonstrated that one-year after transplantation, TheraCyte device was biologically inert and lacked adverse effects, when transplanted in humans. Nevertheless, marked fibroblast overgrowth occurred and almost all the tissues were fibrotic (Tibell et al. 2001). A further study has reported that an immortalized cell line derived from human islets (betaLox5), and induced to differentiate, formed long-term survival and functional POs also immune-protected when allocated in TheraCyte devices (Itkin-Ansari et al. 2003). After that, some preclinical studies were carried on to test the TheraCyte device housing rat islets (Sörenby et al. 2008), human islets and human fetal pancreatic POs (Lee et al. 2009). Sörenby et al. (Sörenby et al. 2008) improved the efficacy of the TheraCyte system by transplanting rat islets in preimplanted TheraCyte devices. Briefly, empty and capped devices were first subcutaneously pre-implanted on the back of athymic mice before diabetes induction. Subsequently, the islets were transplanted in the already implanted and newly pre-vascularized device. Following these modifications, an improvement of PO efficacy was obtained. Moreover, the preimplantation procedure significantly reduced the number of macroencapsulated islets required to restore normoglycemia (Sörenby et al. 2008), without a detectable T-cell response (Lee et al. 2009). It is important to remark that  $\beta$ -cells at this stage of maturation may avoid the immune T-cell response (Lee et al. 2009).

In vivo differentiation into predominantly  $\beta$ -cells was also obtained when huESC-derived endoderm cells were seeded into the TheraCyte

device (Motte et al. 2014). Biosafety was supported by the absence of increased biomass or hESC cell escape for up to 150 days (Kirk et al. 2014). However, an accumulation of CD8<sup>+</sup> T cells surrounding the membrane was observed in some of these devices (Boettler et al. 2016), indicating a certain grade of immune reaction against the graft.

In vitro PEGylation maintains viability and insulin secretory capabilities of transplanted islets (Lee et al. 2002), but also protects them from cytokines secreted by immune cells (Lee et al. 2004). Moreover, PEGylated-macroencapsulation allows a stable blood glucose level of the allotransplants after one year (Lee et al. 2006a). Since PEGylation makes transplanted POs protected against the recipient immune system, a reduction of cyclosporine A dosage was requested (Lee et al. 2006b), and a reduced graft immune infiltration was detected (Lee et al. 2006c). The use of a combination of immunosuppressive drugs was associated with better outcomes (Im et al. 2013). Coated thin PEG-layers on islet surface named “Layer-by-layer (LbL) PEGylation” have been shown to allow a further reduction of islet volume per unit, which facilitates the exploitation of the portal vein for transplantation (Wilson et al. 2008).

### 4.3 Drug-Charged Devices/Scaffolds

The use of dexamethasone-charged macroporous scaffolds has been proposed to inhibit the host immunoreaction. This original approach was developed to accelerate islet engraftment by promoting the expansion of the anti-inflammatory M2 macrophages (Jiang et al. 2017). To generate favorable host responses and to improve the overall outcomes of the transplant polydimethylsiloxane (PDMS)-based 3D scaffold platform, a local and controlled delivery of dexamethasone (Dex) was used (Jiang et al. 2017). In particular, Dex-scaffold accelerated islet engraftment in a diabetic mouse model, improving glucose control early after transplantation. Remarkably, it was demonstrated that lower doses of Dex (0.1% or 0.25%) were

able to induce a M2 phenotype of macrophages interfering with inflammation during the first post-implantation week, whereas higher doses of Dex (0.5% and 1%) significantly delayed the engraftment and function of islets (Jiang et al. 2017).

### 4.4 Vascularization-Enhanced Macroencapsulation

The use of immune-isolated macrodevices designed to islet delivery into extrahepatic transplant site is not limited to synthetic PEG-based hydrogel macrodevices. In fact, a two-component synthetic PEG hydrogel macrodevice system has been generated. These PEG-devices consist in a hydrogel core cross-linked with a non-degradable PEG-dithiol and a proteolytically sensitive vasculogenic outer layer to allow matrix degradation and to enhance vessel infiltration (Weaver et al. 2018). These PEG-dithiol devices promoted engraftment and overall graft efficacy, resulting in enhanced vascular density and in the improvement of islet viability when transplanted in diabetic rats (Weaver et al. 2018).

Through 3D printing technology, Farina et al. (2017) designed an innovative and refillable encapsulation system transcutaneously implanted. The PLA-system generated a prompt and efficient PO vascularization, and supported the long-term graft survival (Farina et al. 2017). Nevertheless, in this first study the in vivo experiments were performed in immunodeficient mice, which did not allow the evaluation of their efficacy in preventing the host immune attack (Farina et al. 2017). More recently, the same group developed a new PLA encapsulation system named neovascularized implantable cell homing and encapsulation (NICHE) that seems to be a promising alternative approach due to their ability to maintain PO viability and a robust hormone secretion (Farina et al. 2018). Despite NICHE system has provided promises to improve long-term viability and function using Leydig cells (Farina et al. 2018), its feasibility in POs still requires validation.

The pro-angiogenic and immunomodulatory effects of the local delivery of the immunomodulating drug fingolimod (FTY720) allow a better oxygenated and tolerant environment for islet engraftment (Bowers et al. 2018). FTY720 is a small molecule that activates sphingosine-1-phosphate receptors and is involved in the regulation of immunomodulatory and pro-angiogenic signals. Based on this concept, Bowers et al. (Bowers et al. 2018) developed a new encapsulation membrane for islet transplantation outside the portal circulation. The FTY720-releasing nanofiber-based semi-permeable membrane increased PO revascularization, and was also able to block the immune response by reducing the number of macrophages and their released cytokines (Bowers et al. 2018).

A similar strategy which can facilitate PO transplantation and preserve their function in extrahepatic sites is represented by the encapsulation of pancreatic islets in synthetic saccharide-peptide (SP) hydrogels (Liao et al. 2013). The SP hydrogel is constituted by natural blocks of amino acids and saccharides, nontoxic and entirely biodegradable (Chawla et al. 2011). The SP hydrogel also displays several exclusive properties, such as ease of handling, crosslinkable at mild physiological conditions, biocompatibility, and *in situ* polymerization after injection (Chawla et al. 2011). The SP hydrogel allows PO survival and function of normal islet structure, but also promotes a rapid vascularization (Liao et al. 2013). Furthermore, it has been demonstrated that SP hydrogels are also able to induce minimal inflammatory cell infiltration. However, they tend to degenerate (Liao et al. 2013).

#### 4.5 Removal/Retrievability

Despite the huge efforts in improving the encapsulation technology, the major problem still relies on the removal (Scharp and Marchetti 2014). This raises significant concerns since this can be associated with transplant failure and clinical complications (Desai and Shea 2017). Retrievability is also an important issue for the regulatory approval processes (Matsumoto et al.

2016). Thereby, efforts are currently focused to generate alternative encapsulation systems readily scalable and conveniently retrievable, also allowing the delivery of a sufficient cell mass. Innovative approaches so far developed to generate easy retrievable devices consist in a cyto-compatible enzymatic approach based on the addition of MMP (Amer et al. 2015). Alternatively, a highly wettable and Ca<sup>2+</sup>-releasing nanoporous polymer, possessing particular mechanical properties which facilitates handling and retrieval of transplanted islets has been generated (An et al. 2018). Innovative retrievable encapsulation system has been also recently developed by An et al. (An et al. 2018). In particular, the thread-reinforced alginate fiber for islets encapsulation (TRAFFIC) consists in a one-step *in situ* cross-linking alginate hydrogel around nanoporous wettable, and Ca<sup>2+</sup>-releasing polymer thread. TRAFFIC has the advantage to provide the essential physical space and biocompatibility for islet transplantation, similar to conventional hydrogel capsules, but displaying mechanical strength enabling the easy handling, implantation, and retrieval (An et al. 2018). Furthermore, due to their particular design, this device may be extended to clinic (An et al. 2018). The therapeutic potential of TRAFFIC has been provided by the restoration of glucose control and immune protection of transplanted human or rat islets in diabetic mice, rats and dogs (An et al. 2018). Furthermore, rapid retrievability can be obtained through a simple laparoscopic procedure (An et al. 2018). The fast and minimally invasive retrievability makes TRAFFIC a promising encapsulation system with a great scale-up potential. Nevertheless, the feasible application in clinic, and particularly in T1DM, has to be evaluated.

#### 4.6 ECM-Enriched Macroencapsulation

Islets in the pancreatic tissue are naturally surrounded by a capsule mainly composed by collagen type I, IV and laminins (Stendahl et al. 2009). Llacua et al. (2016) demonstrated

the ability of specific ECM components (collagens or synthetic laminin peptides) to support survival and function of encapsulated human islets (Llacua et al. 2016). This technology was further applied to evaluate the immunoregulatory effects of capsules covered with a mix of artificial ECM (A. ECM) and Layer-by-Layer (LbL) PEGylation (A. ECM + PEGylation) in non-human primate xenografts (Andrades et al. 2008; Haque et al. 2017; Llacua et al. 2018). The authors demonstrated that ECM-enriched encapsulation system acts as an effective immune barrier, translating in the increase of xenograft survival in the absence of immunosuppressive therapy (Llacua et al. 2018). Although the ECM-enriched encapsulation system is also able to protect POs against the host immune response and to prolonge islet cell survival, its ability to induce islet re-vascularisation remains to be addressed. According with this concept, further studies have been performed to improve PO vascularization. In particular, vascularization improvement was obtained by incorporating heparin in nanofilms containing star-shaped polyethylene glycol (starPEG) (Lou et al. 2017) or silk fibroin macroporous (SF)-scaffolds (Mao et al. 2017). Additional anti-inflammatory and anti-coagulant properties along with the improvement of intra-islet vascularisation have been obtained by enriching capsules with glycosaminoglycans and heparin (Hep-PEG) (Lou et al. 2017). Heparin-mediated vascular endothelial growth factor (VEGF) binding, resulting in the activation of the endogenous VEGF/VEGFR2 pathway, has been reported to mediate endothelial proliferation and re-vascularisation (Lou et al. 2017). These mechanisms have been also previously described for both non-encapsulated (Cabric et al. 2007; Cabric et al. 2010) and encapsulated human islets (Marchioli et al. 2016). Given the pro-angiogenic, pro-survival and minimal post-transplantation inflammatory reaction, as well as the possibility to introduce different trophic factors, Hep-PEG-based encapsulation system is considered a very promising approach for clinical implementation.

#### 4.7 Silk Fibroin-Based Macroencapsulation

The fibrin matrix, due to its good biocompatibility, rapid biodegradability, and easy production, is the most widely investigated natural biopolymeric material (Park and Woo 2018). In particular, the bioactive molecules conjugated with fibrinogen, can promote tissue morphogenesis, cell migration, proliferation, differentiation or maturation after cell adhesion on fibrin matrices (Park and Woo 2018). Thereby, fibrin matrices have been exploited as therapeutic strategies for tissue engineering applications (Park and Woo 2018). These concepts were used for the development of new materials such as autologous platelet- and plasma-derived fibrin scaffolds (Anitua et al. 2019). Synthetic scaffolds based on 3D silk matrices with ECM-derived motifs were also used to promote the aggregation of mouse and human primary cells into functional POs (Shalaly et al. 2016). Afterthought, POs generated by co-encapsulation of islets with MSCs in a silk hydrogel have been shown to increase insulin I, insulin II, glucagon and PDX-1 gene expression. This was associated with a better glucose-induced insulin response (Davis et al. 2012). Diabetic mice implanted with Silk fibroin hydrogel-islet-MSC system showed a prompt return to euglycaemia with a significative reduction of T-helper (Th)1-derived cytokines (Hamilton et al. 2017). Thereby, co-culture of islets and MSCs in a silk-hydrogel-islet system has been proposed to avoid the host immune-attack. However, additional studies are required to demonstrate the stability, the function and the biocompatibility before transfer of this approach to humans.

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## 5 Oxygenated Devices

It has been postulated that chronic effects of non-immunologic factors such as hypoxia and the hyperglycemic milieu could damage the encapsulated islets, resulting in a gradual and short-term loss of efficacy (Safley et al. 2018b).

This prompted researchers to propose new preventing approaches. In particular, major efforts have been focused on generating new methodologies able to increase oxygen supply to POs. Oxygen supply is a crucial issue for cell survival in bioartificial pancreas (BAPs), since poor oxygen supply causes PO central necrosis. Implantation and removal of macrocapsules actually imply minimal risks, but the transport of oxygen and nutrients is still limited. Therefore, new strategies must be developed to improve oxygenation (Hwa and Weir 2018). At this regard, bioengineering research in diabetes mainly focused on the development of new 3D scaffolds able to supply sufficient oxygen and nutrients to POs (Iwata et al. 2018).

The generation of an oxygenated chamber system, based on the incorporation of a refillable oxygen tank ( $\beta$ Air device) into the immunisolating alginate poly-membrane was the first technology developed to improve oxygenation of the encapsulated POs (Ludwig et al. 2012). Indeed, the feasibility to increase the oxygen supply in small and large animal models was demonstrated (Barkai et al. 2013; Neufeld et al. 2013). Based on these promising results, a phase 1 clinical trial was started to demonstrate the efficacy of the  $\beta$ Air (Ludwig et al. 2013). The authors demonstrated that  $\beta$ Air inhibits inflammation of human islets implanted in the absence of immunosuppression. Additionally, an increased vascularization and the enhancement of the oxygen supply were reported (Ludwig et al. 2013). A second phase 1 clinical trial demonstrated that  $\beta$ Air device was safe and able to enhance survival of allogeneic islets (Carlsson et al. 2018). Nevertheless, the real benefits obtained by the  $\beta$ Air device were limited, due to the low increase of circulating C-peptide and the lack of a real impact on the metabolic control (Carlsson et al. 2018). Additionally, the formation of fibrotic tissue, the presence of immune cells, and the deposition of amyloid were also detected in the endocrine tissue (Carlsson et al. 2018).

A new generation of 3D scaffolds containing oxygen generators have been proposed as alternative technology to improve PO oxygenation. Different approaches have been proposed. The new

devices included, (1) polydimethylsiloxane encapsulated solid calcium peroxide (PDMS-CaO<sub>2</sub>) (Pedraza et al. 2012), (2) active microorganisms such as *Synechococcus lividus*, which photosynthetically generated oxygen (Evron et al. 2015), and (3) SPO and CPO (Lee et al. 2018). These studies demonstrated a successful enhancement of oxygenation that translated into a reduced hypoxia-induced cell loss during the precarious vascularization period (Pedraza et al. 2012). These beneficial effects resulted from the capability to supply oxygen at high cell loading densities (Pedraza et al. 2012), with an average of 0.026 mM/day oxygen, for more than 6 weeks (Evron et al. 2015). This prevented the development of detrimental oxygen gradients in the core of large implants. The beneficial effects of oxygen-generating scaffolds were further supported by the studies of Lee et al. (2018) who used neonatal pancreatic cell-clusters (NPCCs). The application of oxygen-generating microparticles (MP) included in a fibrin-conjugated heparin/VEGF and implanted in streptozotocin-induced diabetic NOD mice was also investigated (Montazeri et al. 2016). The MP improved the POs engraftment and function, the blood glucose control, body weight, glucose tolerance, serum C-peptide, and graft revascularization, by reducing the islet mass necessary to obtain this goal (Montazeri et al. 2016). It became evident that oxygen-generating materials such as sodium percarbonate (SPO) and calcium peroxide (CPO) could be efficient in supplementing oxygen to transplantable naked and encapsulated islets in diabetic patients (McQuilling et al. 2017). Indeed, it has been shown that SPO and CPO can be used to improve islet viability and function. Nevertheless, additional studies are required to control the oxygen generation rate able to provide the suitable oxygen supply over an extended period of time avoiding tissue damage resulting from oxidative stress (McQuilling et al. 2017). Of note, it has been suggested that this technology could be also applied, as a new animal-free experimental scaffold platform, to study the interactions between pig islets and human blood components *in vitro* (Lee et al. 2018).

### 5.1 Perfluorocarbons (PFCs) as Oxygen Carriers in POs

PFCs are obtained by fluorine replacement of hydrogen atoms in hydrocarbons (Hosgood and Nicholson 2010). The PFCs have a higher density than water and a high capacity to dissolve oxygen. Carbon-fluorine bonds are very long and render the molecule biologically and chemically inert (Hosgood and Nicholson 2010). Due to their lipophilic nature and their high oxygen solubility, PFCs have been largely explored to improve cell and tissue oxygenation. They can be also adapted and used as blood substitutes for the treatment of cardiac ischemia, anemia, and organ preservation (Hosgood and Nicholson 2010). However, controversial results have been reported. Bergert and collaborators (Hosgood and Nicholson 2010) have demonstrated that, compared to control rat islets, PFC-cultured islets showed a significant increase of DNA fragmentation and a reduced glucose sensitivity. On the contrary, other studies indicated that, although advantageous to transfer human harvested organs, the use of PFCs may be comparable to conventional islet culture protocols used for transplantation (Bergert et al. 2005). A different study demonstrated that perfluorotributylamine (PFTBA) emulsion co-encapsulated insulin secreting cells ( $\beta$ TC-tet insulinoma cells) did not have additional effects in terms of viability or function (Goh et al. 2010). Nevertheless, it was suggested that PFCs could be potentially applied as a culture method to improve islet preservation, in particular for pancreatic islets isolated from marginal pancreata (Ricordi et al. 2003). Additional studies are still required to demonstrate the biological benefits of PFCs on POs.

### 5.2 Generation of Hypoxia-Resistant Islets

It became evident that, while encapsulation clearly protects POs against the host immune attack, it impairs PO survival and long-term function. VEGF plays a crucial role in  $\beta$ -cell function and islet regeneration (Nikolova et al. 2006; Jabs

et al. 2008). Moreover, it has been reported that a splice variant-1 of the GHRH receptor expressed in human pancreatic islets was able to generate antiapoptotic signals by modulating VEGF levels and by inducing angiogenesis (Ludwig et al. 2010). “Hypoxia-resistant islets” have been generated using implanted devices containing pancreatic islets and GHRH agonists (JI-36) (Ludwig et al. 2012). A significant hypervascularization and enhancement of the graft function, associated with a parallel improvement of glucose tolerance and increase of the  $\beta$ -cell insulin reserve were observed (Ludwig et al. 2012). This also allowed a reduction of the islet mass required for metabolic control (Ludwig et al. 2012). However, even if promising, the feasibility of this innovative technology as alternative xenotransplantation approach requires further validation.

### 5.3 Mathematical Models

As extensively documented, the main goal to improve biotechnological approaches is an efficient oxygenation, allowing a long-term survival and function of newly generated POs. One of the most relevant challenges to deal with relies on the thickness of the cell layer (Wu et al. 1999). In encapsulated POs, the inner core mimics pancreatic islets while the peripheral shell acts as an inert defense. Oxygen consumption by the cells is a hurdle that follows two rules, (1) the limited diffusion, and (2) the limited consumption. Using mathematical models it has been suggested that hypoxia directly depends on the PO radius and would likely occur in high cell density conditions (King et al. 2019). According with this model, a better viability should be obtained with a PO radius around 142  $\mu$ m or fewer, and with an encapsulating radius lower than 283  $\mu$ m (King et al. 2019).

The effectiveness of supplementary oxygen suppliers in a  $\beta$ -cell 3D culture has been estimated by calculating the spatio-temporal distribution of oxygen concentrations inside the scaffolds (McReynolds et al. 2017). Two different types of simulations (constant cell density and cell growth simulation) used in this work, have lent



a hand to determine the effectiveness of oxygen-releasing microbeads (McReynolds et al. 2017). Briefly, hydrogen peroxide was encapsulated into nontoxic PDMS disks which provided oxygenation to  $\beta$ -cell cultures (McReynolds et al. 2017). Detection of the spatial-temporal distribution of oxygen tension inside a scaffold would be a fascinating approach to be pursued in the future. McReynolds and collaborators (McReynolds et al. 2017) have also suggested that optimization of the cell culture conditions, including cell seeding density and time of culture, are critical issues.

As widely described, the new 3D devices are mostly based on oxygen diffusion. Nonetheless, mathematical models indicate that convective transport should be a more efficient approach for the oxygen transfer (Iwata et al. 2018). Theoretical studies have described how oxygen supply in BAPs could predict the necrosis area in the islets (Iwata et al. 2018). Thereby, according with these models, to correct the difference in pressure between the device and the site of implantation, the BAPs should be directly connected to the blood vessels (Iwata et al. 2018). Platelet deposition usually takes place in implants subjected to blood flow, and eventually leads to thrombus formation (Affeld et al. n.d.). Thus, BAP coated with blood compatible materials could prevent thrombus formation in long-term BAPs.

In summary, despite enormous efforts devoted to the encapsulation technology, oxygenation remains the main challenge in diabetes bioengineering. Mathematical models, established that an oxygen-enabled 3D culture system should effectively provide an improved oxygen distribution within the scaffold, allowing an enhanced secretion of insulin from cells. Nevertheless, the application in humans is still missing.

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## 6 Vascular Improvement in Transplants

Pancreatic islets are extremely vascularized structures by a capillary network which is critical for glucose sensing and for providing nutrient

supply and rapid secretion of hormones into the blood stream (Richards et al. 2010). Islets with an altered vascularization do not properly regulate glycemia (Lammert et al. 2003), indicating that engineered islets should permit an efficient mass transport and well-organized vascularization. This requires the presence of a porous implantable construct allowing vessel infiltration. Recent data indicate that the range of pore size that maximizes vascularization is narrow and corresponds to 30–40  $\mu\text{m}$ , (Madden et al. 2010). This suggests that an accurate control of the pore size would significantly favor vascularization of implantable scaffolds (Buitinga et al. 2017). It has been clearly demonstrated that the lack of an adequate revascularization (Smink et al. 2017), the recurrence of autoimmunity (Piemonti et al. 2013), the prompt blood-mediated inflammatory response (Kourtzelis et al. 2016; Samy et al. 2018), the ischemic injury (Faleo et al. 2017), and the activation of Natural Killer (NK)T cells (Saeki et al. 2017) are the main factors contributing to the engraftment failure.

Approaches exploiting angiogenic factors have been used in tissue engineering. Thus, encapsulation devices enriched in pro-angiogenic molecules such as VEGF, to improve PO vascularization is strongly encouraged (De Rosa et al. 2018; Schweicher et al. 2014). Trivedi et al. (2000) have proposed an alternative approach to solve the deficiency of vascularization in devices using a double layer capsule formed by three different layers combined with a transcutaneous infusion of VEGF. This approach leads to a reduced delay of the diffusion time due to the development of a new vascular network through large pores. The authors also showed that small pores could protect POs from the immune attack (Trivedi et al. 2000). However, even if a reduction in glycemia was detected, normoglycemia was never achieved in transplanted diabetic mice (Sweet et al. 2008). The authors have suggested that this unexpected result, rather than to the device itself, may be related to the number of implanted islets, corresponding to the half of islets usually transplanted into T1DM patients (Sweet et al. 2008).

Alternative studies applied a semi-interpenetrating polymer network (SIPN) as subcutaneous implantable carrier to deliver cells and to allow vascularization (Mahou et al. 2017). SIPN was generated by reacting a blend of vinyl sulfone-terminated polyethylene glycol (PEG-VS) and PMAA-Na with dithiothreitol (Mahou et al. 2017). This combination allowed the formation of vessels in the surrounding tissues, which is 2–3 times superior to that obtained with PEG alone (Mahou et al. 2017). The biological activity of the SIPN in glucose control was demonstrated in diabetic mice (Mahou et al. 2017).

Re-endothelization represents an innovative approach designed to solve the issue of blood supply. Endothelial progenitor cells (EPCs) have been used to generate pre-vascularized scaffolds. The main advantage identified was the direct connection between the pre-vascularized host blood circulation and the newly formed blood vessels (Guo et al. 2018). Very recently, Skrzypek et al. (2018a) developed a functional and not degradable flat polyethersulfone/polyvinylpyrrolidone (PES/PVP) porous membrane to create an early microvascular network without the addition of hydrogels (Skrzypek et al. 2018a). Applying this technology, pre-vascularized devices could be obtained by co-culturing different cells (Skrzypek et al. 2018a, b; Groot Nibbelink et al. 2018). It was demonstrated that the particular surface topography obtained with this method enhanced the formation of a stable vascular network in the membranes. Furthermore, the *in vitro* pre-vascularization allowed a faster connection of POs with the host vasculature, impacting on the engraftment, and on the long-term implant survival and function (Skrzypek et al. 2018b).

Oligomers have been used for tissue engineering approaches in T1DM pre-clinical models (Stephens et al. 2018). The feasibility of collagen I oligomeric-based macroencapsulation was already demonstrated (Stephens et al. 2018). Collagen-based macroencapsulation was able to improve mouse islet function and longevity (Stephens et al. 2018). Cytoarchitecture,

revascularization, and immunetolerance were also demonstrated (Stephens et al. 2018). Furthermore, STZ-induced diabetic mice showed a rapid glycemic control and a sustained normoglycemia until 40 days post-transplantation (Stephens et al. 2018).

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## 7 Natural Extracellular Matrices as Biological Scaffolds

Undoubtedly, scaffolds displaying features similar to native islet extracellular matrix (ECM) would most likely succeed. The ECM is synthesized by cells of each tissue and provides a physical niche for cell attachment. ECM composition includes polysaccharides and proteins, such as collagen and elastin (Theocharis et al. 2016). One of the most recent technologies enabling the isolation of native ECM is the organ decellularization (Badylak et al. 2011). ECM derived from decellularized organs has been recently used for tissue engineering (Guruswamy Damodaran and Vermette 2018a). Decellularized scaffolds have been proposed as a source of native ECM exploitable in a wide-ranging regenerative medicine applications (Celikkin et al. 2017). ECM offers structural and function support and acts as a substrate for cell migration. The ECM mechanical behavior depends on its physical properties such as insolubility, porosity, rigidity, and its topography (Celikkin et al. 2017). The ECM components are very conserved through different species, and include fibers, collagens, proteoglycans, glycoproteins, and mucins (Brown and Badylak 2015). ECM-scaffolds respond and release growth factors, that modulate the immune response, and allow the recruitment of progenitor cells (Swinehart and Badylak 2016).

Islets embedded in the acinar tissue of the pancreas represent only the 1%–2% of pancreatic mass (Jansson et al. 2016; Aamodt and Powers 2017). They are spherical clusters of cells highly innervated and vascularized, containing ECM which coordinate and support cellular survival, proliferation and differentiation (Irving-Rodgers

et al. 2014; Aamodt and Powers 2017; Cheng et al. 2011; Kuehn et al. 2014; Alismail and Jin 2014). In islets the ECM regulates multiple aspects of cell function, including insulin secretion, proliferation, survival, and participates to the preservation of spherical morphology (Stendahl et al. 2009). Pancreatic decellularized scaffolds play an essential role in regenerative medicine and represent a step toward the development of bioengineered pancreas. As other natural bioscaffolds, pancreatic decellularized scaffolds retain the native 3D architecture, the vasculature, the ductal channels, and the pancreatic ECM composition (Guruswamy Damodaran and Vermette 2018b). The interaction between cells and scaffolds improves pancreatic islet cell survival and insulin production potentially used for regenerative purposes (Goh et al. 2013).

Bioscaffold plasticity has been also demonstrated by using stem cells, which received and responded to the environmental signals. In this context mechanical forces play a central role in the regulation of multiple functions (Rana et al. 2017). Indeed, the adhesion of cells to ECM, the formation of cell-cell junctions, and of a mechanoreponsive cell cytoskeleton, are critical features in stem cell biology (Rana et al. 2017), particularly in cell fate determination (He et al. 2018). Plasticity and compatibility of ECM scaffolds derived from different species have been also demonstrated (Chaimov et al. 2017). Decellularized pancreatic scaffold biocompatibility in the absence of toxicity has been also confirmed (Chaimov et al. 2017). Particularly, Chaimov and collaborators (Chaimov et al. 2017) developed an innovative microencapsulation platform based on solubilized whole porcine pancreatic ECM mimicking the original decellularized ECM (Chaimov et al. 2017). This natural fibrous 3D niche was able to support the viability of pre-differentiated cells to promote differentiation and to improve insulin secretion (Chaimov et al. 2017). Moreover, these re-cellularized scaffolds were non-immunogenic and were able to improve the glycemic control in diabetic mice (Chaimov et al. 2017). It has been reported that growth factor-enriched decellularized pancreatic scaffolds induce differentiation of

mouse pancreatic stem cells (PSCs) into pancreatic  $\beta$ -like cells (Wan et al. 2017). Bioscaffold-induced differentiation, proliferation and insulin secretion, occur independently of scaffold-derived tissue (Zhou et al. 2016). This suggests the existence of a dual interplay between the ECM and the cell.

Decellularized pancreata show the characteristic homogeneous porous structure (Hashemi et al. 2018). Thus, to solve the problem of PO blood supply after *in vivo* transplantation, bioengineering has taken advantage of this valuable feature to enhance scaffold vascularization by co-seeding POs with EPCs (Guo et al. 2018). Newly formed blood vessels (Guo et al. 2018) and improvement in functional preservation characterized the re-endothelized scaffolds (Chaimov et al. 2017). Despite these promising results, vascularization still remains a challenge to generate a functional equivalent ECM-based pancreata for transplantation.

Generation of acellular pancreatic scaffolds from small animals represents a relevant issue for future pre-clinical studies. The next and most closely need is represented by the large-scale production. Some recent studies demonstrated the feasibility (Berman et al. 2016; Katsuki et al. 2016). Nonetheless, despite the improvement of islet metabolic function and the significant preservation of the islet architecture and the intra-insular vascularization (Berman et al. 2016; Katsuki et al. 2016), long-term studies and implantation *in vivo* are still needed. Moreover, it was recently described that signals promoting cell differentiation could be heterogeneous in nature, depending on the type of organ, and on the type of tissue from which they derive (Brown and Badylak 2015). For example, it has been shown that HLSC seeded in liver decellularized scaffolds can differentiate into three different cell types (hepatocyte-, endothelial- and epithelial-like cells) (Navarro-Tableros et al. 2015). This indicates the high ECM plasticity. Therefore, the use of ECM bioscaffold-based technology could be exploitable for hetero-organ approaches. This possibility has been recently validated by Vishwakarma and collaborators (Vishwakarma et al. 2018). Indeed, they were able to induce

differentiation of human hepatic progenitor cells (hHPCs) into insulin-progenitor cells when hHPCs were seeded into xenogeneic rat acellular spleen in the presence of specific trophic factors and hyperglycemic environment (Vishwakarma et al. 2018). These data further support the hypothesis (Navarro-Tableros et al. 2015; Vishwakarma et al. 2018), that cell commitment is a complex process, modulated in part by the nature of the scaffold, but also by the type of hosted cells. In fact, the study of Vishwakarma et al. (2018) suggests that natural bioscaffold properties should be further investigated for regenerative approaches. Finally, pancreatic scaffolds, used in most if not all studies, have been generated from “healthy” tissues. Nevertheless, there are not definitive data on the feasibility to use “sick” organs for clinical applications. A very recent study, aimed to evaluate the feasibility at using diabetic pancreata to generate ECM for regenerative purposes (Huang et al. 2018a), demonstrated that similar to healthy scaffolds, those obtained from T1DM and T2DM pancreata preserved their ECM composition, 3D ultrastructure and released cytokines (Huang et al. 2018b). Nevertheless, the biological activity of this “diabetic scaffolds” has been not yet tested. These important results, even if preliminary, support the idea that marginal organs could serve as alternative sources of natural ECM scaffolds. Thereby, the application of marginal tissues could be implemented in future regenerative approaches in order to strongly support this hypothesis.

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## 8 CRISPR/Cas9 Genome Editing Technology to Enhance Insulin-Producing Cell Function

The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) combined with the DNA endonuclease Cas9 (CRISPR-Cas9) has been considered an innovative application which

could completely change both medical and biotechnology approaches in our century. Using short guide target-specific sgRNAs, the Cas9 can be directed to any genomic location by inducing double strand breaks to allow non-homologous end joining or homologous recombination of the genome at specific sites (Kim et al. 2017). CRISPR works together with *Cas* gene to cleave genetic material, thus opening the possibility to use CRISPR-based approaches to induce  $\beta$ -cell differentiation (Gerace et al. 2017). Other approaches different from CRISPR, are mainly based on viral-mediated transfer of key pancreatic transcriptional factors such as *Pdx-1* (Karnieli et al. 2007), *NeuroD1* (Kojima et al. 2003) to either somatic or adult stem cells such as MSCs (Gerace et al. 2017). However, although CRISPR-Cas9 system is widely applied in a number of research fields (Kim et al. 2017), its application to generate or to enhance the function of insulin-producing cells is still limited. At this regard, Giménez et al. (2016) applied the CRISPR-ON system to activate the endogenous human insulin gene (*INS*), obtaining a significant upregulation of the insulin mRNA expression when the dCas9-VP160 construct and four sgRNAs (targeting the proximal *INS* promoter) were co-transfected in T1DM patients-derived skin fibroblasts (Giménez et al. 2016). More recently, one homozygous ATG > ATA mutation at codon 1 of the insulin gene was reverted to wild-type ATG in hiPSC by using CRISPR/Cas9 technology (Ma et al. 2018). The insulin mRNA expression and hormone secretion confirmed the functional correction (Ma et al. 2018). Moreover, the modified  $\beta$ -cells were able to reverse diabetes in STZ-diabetic mice (Ma et al. 2018). This work has opened the possibility to combine gene and cell therapy to restore glucose homeostasis in non-immune-mediated diabetic setting. As a future perspective, the combination of CRISPR-Cas9 and encapsulation technology may contribute to improve current methodologies to generate long-term functional POs to be applied in diabetes.

## 9 3D Bioreactor Systems to Produce and Maintain Insulin-Producing Cells

Although stem cells hold the promise to generate endless insulin-producing cells, most of the current preclinical protocols have been performed in lab-scale (Navarro-tableros et al. 2018; Kim et al. 2016a; Wu et al. 2007). Hence, the implementation of alternative culture systems, able to generate a large number of functional insulin-producing cells, is still needed. Cell culture in 3D bioreactors has been used for several decades by the biopharmaceutical industry (Abu-Abisi et al. 2014), since they allow a tight control of relevant parameters such as pH, temperature and gas supply, and guarantee a more efficient cell expansion and a higher cell viability and function (Petry et al. 2018). In this chapter, we will provide a general overview of different 3D bioreactors that have been employed to generate and/or maintain viable and functional POs obtained from different sources.

### 9.1 3D Bioreactors Used for Insulin-Producing Cell Cultures

**Spinner Flasks (SF)** SF, which are currently considered the simplest form of 3D bioreactors, consist in a magnetic stirrer-integrated flasks that provide a turbulent fluid flow environment to partly alleviate limitations of nutrient and oxygen diffusion (Ratcliffe and Niklason 2002) (Fig. 1a). However, SF suffer from similar drawbacks as classical 2D culture systems in terms of requirements of individual handling and scale-up (Ratcliffe and Niklason 2002), and to the best of our knowledge despite these limits, SF are the most commonly used bioreactors in the field of  $\beta$ -cell research.

In the early 2000s, Blyszczuk and collaborators (Blyszczuk et al. 2003) developed a 10 days culture protocol in SF (30 rpm agitation at 37°C and 5% of CO<sub>2</sub>) to induce differentiation of mouse embryonic-derived nestin-positive progenitors into immature insulin-positive POs

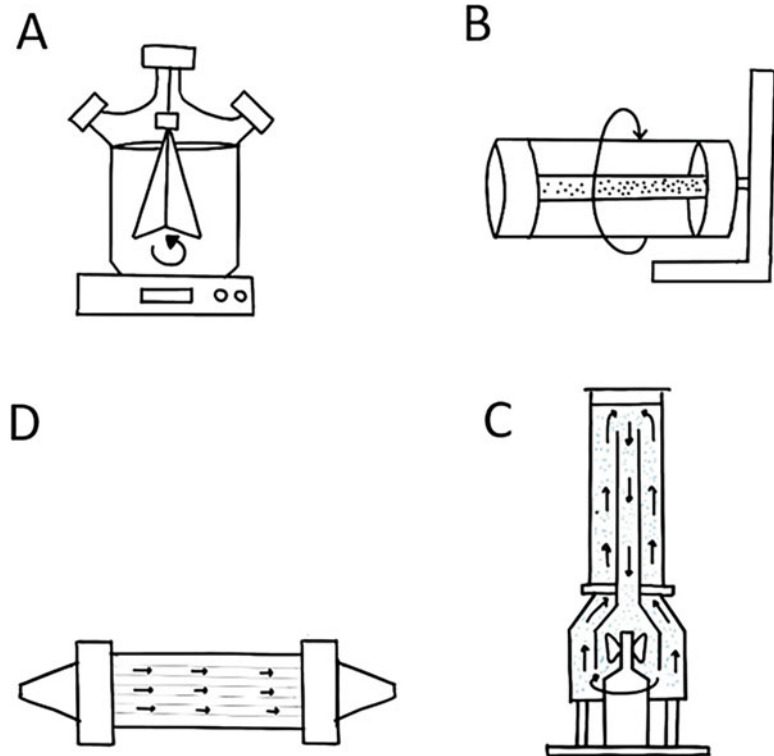
(Blyszczuk et al. 2003). Transplantation of these POs was able to ameliorate the glycemic control in immunocompromised STZ-diabetic mice (Blyszczuk et al. 2003). More recently, hESCs, initially maintained in 500 mL SF (70 rpm agitation at 37°C and 5% of CO<sub>2</sub>) and subsequently cultured in standard 2D plates in presence of specific growth factors, were found to successfully differentiate into POs able to restore normoglycemia in diabetic mice (Pagliuca et al. 2014). Long-term glycemic control was obtained in diabetic mice, when encapsulated hESC-derived POs were pre-cultured for 24 h in SF, before their implantation (Vegas et al. 2016).

Chawla and collaborators (Chawla et al. 2006) developed a new SF system to generate POs in a large scale. To this end, porcine pancreatic neonatal endocrine tissues, at different densities, were seeded in SF (100 rpm agitation at 37°C and 5% of CO<sub>2</sub>) in serum-free media with a specific differentiation-cocktail (Chawla et al. 2006). Using this approach, and after 9 days of culture, a large number of POs containing insulin-positive cells were produced. In addition, the POs responded to glucose and contained all islet cell types (Chawla et al. 2006). More recently, Lock et al. (2011) demonstrated the superiority of SF in the generation of mouse cell line (MIN6)-derived POs compared to the 2D standard culture. The POs generated in SF (60–100 rpm agitation at 37°C and 5% of CO<sub>2</sub>) were associated with a 20% higher viability (64 vs 84%), a more regular shape morphology and the ability to grow for at least 2 weeks (Lock et al. 2011). In addition, the POs generated in SF, were more similar to native islets, in terms of ultrastructure, glucose-insulin secretion, incretin expression and were characterized by a lower necrosis grade (Lock et al. 2011).

**Microgravity Bioreactor (MB)** The original version of the MB was developed by NASA engineers and patented in 1990 under the form of rotating wall vessels (RWV), also known as high aspect ratio vessels (HARV) (Schwarz et al. 1992) (Fig. 1b). MB provided a continuous circular rotation of media and allowed cultured cells to

**Fig. 1 Schematic representation of different bioreactor types used to generate and maintain insulin-producing cells.**

(a) Spinner Flasks (SF). (b) Rotating wall vessels (RWV). (c) Hollow fiber membrane bioreactor (HFB). (d) Fluidized-bed bioreactor (FBB)



be suspended in a low gravitational field (free fall), characterized by a low turbulence and hydrodynamic shear stress coupled with a high mass of oxygen transfer (Daoud et al. 2010). The first study involving MB and islets was carried out by Cameron and collaborators (Cameron et al. 2001) in 2001. To facilitate islet engraftment after transplantation, they co-cultured porcine neonatal islets with Sertoli cells (isolated from rat testis) in MB for up to 14 days. After 4 days in MB culture, large Sertoli-islet POs were formed (0.5–3 mm diameter). However, although cellular viability (>90%) and glucose responsiveness was maintained, islets underwent disaggregation into single cells. Primary mouse islets cultured in MB were significantly less immunogenic compared to standard cultured islets (Rutzky et al. 2002). Moreover, to maintain euglycemia throughout 100 days in diabetic animals the number of MB-cultured POs required were 50% less than the non-MB counterpart (Rutzky et al. 2002). Furthermore, ultrastructure studies revealed that

islets appeared healthy and similar to fresh islets (Rutzky et al. 2002). Using MB (8 rpm at 37°C and 5% of CO<sub>2</sub>) the improvement of human islet long-term function and viability was also demonstrated (Murray et al. 2005).

In an elegant work, Tanaka and collaborators (Tanaka et al. 2013) described the generation of mouse  $\beta$ -cell (MIN6 cell line)-derived POs. They loaded a two-compartment culture chamber onto the center of the 3D clinostat to generate microgravity-like conditions. Unlike the RWV, this new designed approach, allowed fresh media flow and gas exchange. In addition, POs had an average size of 250  $\mu$ m and were glucose-responsive (Tanaka et al. 2013).

#### **Hollow Fiber Membrane Bioreactor (HFB)**

HFB utilizes specialized membranes to retain cells inside, and to allow an efficient exchange of nutrients and waste products by the flow of the medium through the lumen (Abu-Absi et al. 2014) (Fig. 1c). The main advantages of the

membrane-based-bioreactors relied on the possibility to obtain an high cell capacity, high volumetric efficiency, and low shear stress (Abu-Absi et al. 2014). However, disadvantages such as a reduced cell viability, membrane fouling and clogging, the lack of product homogeneity have limited their large-scale application (Abu-Absi et al. 2014). Hoesli and collaborators (Hoesli et al. 2009) developed a procedure for immobilization of large-scale cell batches in alginate-filled hollow fiber bioreactors (AHFBRs). This process improved insulin secretion and cell viability of porcine neonatal pancreatic cells (Tanaka et al. 2013) and enhanced the expansion of the insulinoma cell line, INS-1 (Sharp and Vermette 2017; Gundersen et al. 2010).

**Fluidized-Bed Bioreactor (FBB)** In the FBB system, the cells are seeded on microcarriers (e.g. Cytopore or Cytoline) or polymer-microencapsulated (e.g. alginate, collagen) and loaded in a stirred tank which allows the flow of the oxygenated nutrient through the cells (Fig. 1d). This design avoids the formation of local high shear stress and provides an interface suitable for large-scale production (Abu-Absi et al. 2014). The feasibility of FBB for production of encapsulated-POs was previously assessed (Buchi-395 encapsulator). Indeed, it was demonstrated their ability to enhance function, viability and integrity as well as insulin responsiveness of 1% alginate-encapsulated POs (range 180–220  $\mu\text{m}$ ) (Nikraves et al. 2017).

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## 10 *In-vitro* Expansion of $\beta$ -Cells from Adult Human Pancreatic and Hepatic Tissues

hPSCs are a promising source of cells for tissue regeneration due to their unlimited proliferative potential and their capability to differentiate into three germ layers including: ectoderm, endoderm and mesoderm (Zuber and Grikscheit 2018). However, concerns still remain regarding cell differentiation commitment and scalability required for human transplantation approaches

(Jones and Zhang 2016). One of the major advantages of hPSCs compared to other stem cells, relies on their pancreatic origin. Indeed, they originated from the pancreatic islets, which could facilitate their commitment into mature POs (Nelson et al. 2009). Under physiological conditions, the endocrine pancreas (islets) has an extremely low turnover rate (Afelik and Rovira 2017), while under high metabolic demands (pregnancy or obesity), adaptation mechanisms such as cell hypertrophy, increased insulin synthesis and secretion,  $\beta$ -cell self-replication occur (Afelik and Rovira 2017). *In vivo*, the  $\beta$ -cell pool, is homogeneous (Brennan et al. 2007) and persist throughout the life as a “neogenic niche” within pancreatic islets (van der Meulen et al. 2017). Koop et al. (Kopp et al. 2011) demonstrated that after *in vivo*  $\beta$ -cell ablation, endocrine cells do not arise from the ducts, but from early pancreatic progenitors expressing Ptf1a, Nkx6.1, Pdx1 and Sox9 markers (Dor et al. 2004). Additionally, terminally differentiated adult  $\beta$ -cells retaining a significant proliferative capacity and accounting for pancreatic turnover and expansion throughout the life, have been identified (Wei et al. 2006). Pancreatic progenitors do not express hormones in basal conditions but can be induced to differentiate into hormone-expressing islet-like-cells (Dor et al. 2004). Although the hPSC aggregation induces the expression of pancreatic endocrine markers such as Pdx1, MafA and hormones like insulin, glucagon or somatostatin, the mRNA and hormone levels are less expressed than in freshly isolated human islets. They fully differentiated only after implantation in mice (Kroon et al. 2008; Davani et al. 2007).

### 10.1 Pancreatic Islet-Derived Stem Cells

The presence of pancreatic precursor cells in adult human pancreas named pancreatic islet mesenchymal stromal cells (huPI-MSC) have been demonstrated and have been recently considered an alternative cell source to generate insulin-producing cells (Zhao et al. 2007; Joglekar and Hardikar 2012; Wang et al. 2013). These huPI-

MSCs exhibit all the characteristics of bone marrow MSC (BM-MSC), including the ability to suppress proliferation of lymphocyte stimulation. The number of harvested islet equivalents from a single donor varies from 300,000 to 600,000 (Kim et al. 2012). This implies that autologous huPI-MSC could be obtained from a small portion of adult pancreatic islets, including those which are discarded (Kim et al. 2012). These properties make huPI-MSC, an available and potentially exploitable source of adult human stem cells to generate  $\beta$ -cell substitutes for cell therapy in diabetes. From a therapeutic point of view, the use of adult stem cells from discarded human cadaveric islets represents an attractive perspective. It is well accepted that mature adult human  $\beta$ -cells could be significantly expanded *in vitro*, but complex de-differentiation and re-differentiation processes are required (Russ et al. 2008). Expanded human  $\beta$ -cell-derived (BCD) cells, constitute the ~40% of cells of the islet cell cultures, which can re-differentiated in response to a combination of soluble factors (Avnit-Sagi et al. 2009; Russ et al. 2011). However, only a part of these cells undergo re-differentiation (Russ et al. 2009). More recently, a different approach demonstrated that overexpression the miR-375, a miRNA highly expressed during human islet development, boosted adult pancreatic precursor cells to redifferentiate (Avnit-Sagi et al. 2009; Joglekar et al. 2009) into mature islets (Klein et al. 2013; Latreille et al. 2015).

Despite these promising results, further efforts are required for a successfully transability, and large scalability. In line with this concept, Carlotti and collaborators (Carlotti et al. 2010) have demonstrated the feasibility to use pancreatic-derived iPSCs in regenerative medicine. Recent studies, have demonstrated the presence of pancreatic stem/progenitor cell populations, named NIP/Nestin-positive Islet-derived Progenitors (Zulewski et al. 2001), PIDM/Pancreatic Islet-Derived Mesenchymal cells (Gallo et al. 2007) and PHID/Proliferating Human Islet-Derived cells (Ouziel-Yahalom et al. 2006) which are able to generate POs on tissue culture plates.

Very recently, Van der Meulen and collaborators (van der Meulen et al. 2017) identified a population of human immature  $\beta$ -cells, originating by trans-differentiation of  $\alpha$ -cells on a specialized pancreatic “neogenic niche” located at the islet periphery (van der Meulen et al. 2017). These cells express insulin but not other  $\beta$ -cell markers. They are transcriptionally immature and do not sense glucose (van der Meulen et al. 2017). This cell population represents an intermediate stage of  $\alpha$ -cell trans-differentiation into conventional  $\beta$ -cell (van der Meulen et al. 2017). These data suggest the possibility to use this cell population for generation of functional POs. However, additional studies are crucial to demonstrate their potential application in clinic.

The presence of high aldehyde dehydrogenase activity (ALDHhi)-pancreatic stem cells, have been also reported by Loomans et al. (2018). The ALDHhi-derived POs contain insulin-producing cells that also express pancreatic progenitor markers such as PDX1, PTF1A, CPA1, and MYC. Nevertheless, these POs appeared immature and did not restore normoglycemia in diabetic mice (Loomans et al. 2018).

## 10.2 Human Pancreatic Ductal-Cells (PANC-1) and Non-endocrine Pancreatic Cells

It was recently demonstrated that de-differentiation of human pancreatic ductal-cells (PANC-1) promotes their differentiation into endocrine pancreatic cells (Donadel et al. 2017). Formation of POs expressing pancreatic markers such as C-peptide, insulin, pancreatic and duodenal homeobox 1 (PDX-1), Nkx2.2, Nkx6.1 was obtained from PANC-1 cells. The expression of glucagon, somatostatin, and Glut-2 was also observed. This was associated with a decrease in the expression of PANC-1 markers (Cytokeratin-19, MUC-1, CA19-9), supporting their pancreatic commitment (Donadel et al. 2017). However, additional studies are required to evaluate their potential use to generate functional and safe POs.



### 10.3 Biliary Tree-Derived Islet Progenitors

Recently, a new source of islet precursors has been identified in a niche located within the human biliary trees (Wang et al. 2013). This niche is characterized by a network of cells with overlapping commitment (Wang et al. 2013). The biliary tree-progenitor cells showed a high proliferation rate when cultured *in vitro*, under specific conditions (Wang et al. 2013). Moreover, these cells were able to aggregate in POs, resembling neo-islets generated from other cell sources (Wang et al. 2013). The POs generated from these progenitors showed ultrastructural, electrophysiological and functional characteristics, including the response to glucose, similar to other POs. Furthermore, the derived neo-islets were able to improve glucose control in immune-compromised diabetic mice (Wang et al. 2013).

### 10.4 Hepatic Stem Cell Derived Islet-Like Structures

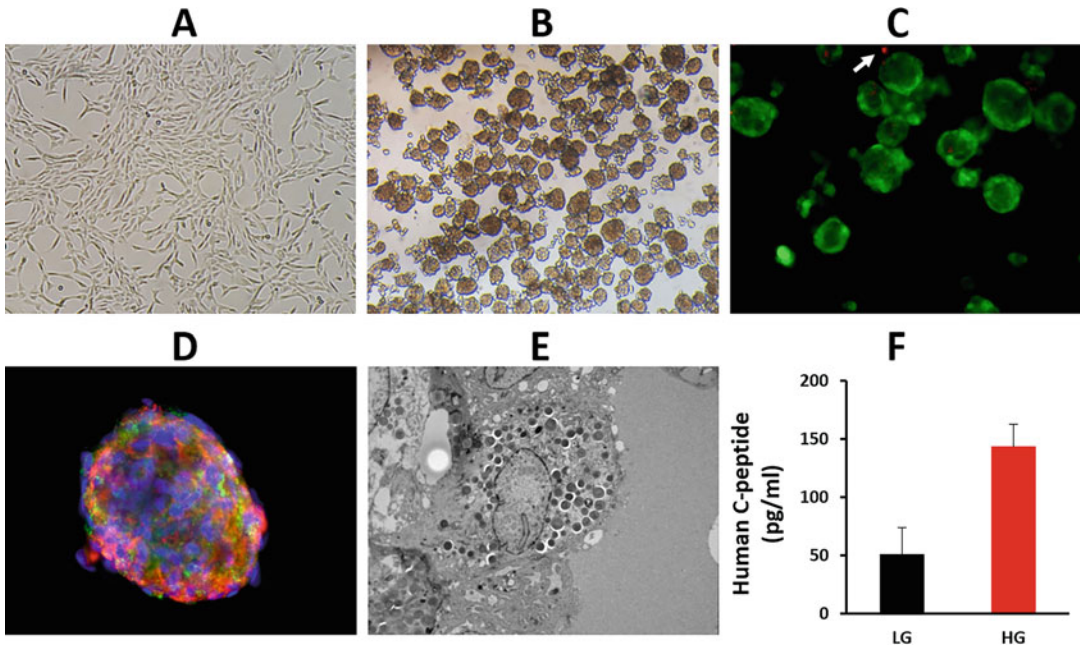
We have recently found that adult human liver stem-like cells (HLSC, Fig. 2a), which are clonogenic and express several mesenchymal and embryonic markers, such as SSEA4, Oct4, SOX2 and nanog, were able to spontaneously generate insulin-producing organoids (Navarro-tableros et al. 2018). This is not surprising since liver and pancreas share common embryonic origins. Charge-induced 3D aggregation of HLSC (Fig. 2b) promoted differentiation into viable insulin expressing cells (Fig. 2c and d) (Navarro-tableros et al. 2018) which contained heterogeneous secretory granules (Fig. 2e). These HLSC-derived POs were able to secrete human C-peptide in response to high glucose concentrations both in static (Fig. 2f) and dynamic conditions (Navarro-tableros et al. 2018). *In vitro* differentiated islet-like structures showed an immature endocrine gene profile but were able to further differentiate after *in vivo* implantation (Navarro-tableros et al. 2018). In fact, when transplanted in non-immunocompetent

diabetic mice hyperglycemia was reverted and the human C-peptide was detectable in mice (Navarro-tableros et al. 2018). As a proof of concept, after removal of implanted islet-like structures diabetes was reestablished, suggesting that diabetes reversal was dependent on the transplanted islet-like structures generated by HLSC differentiation (Navarro-tableros et al. 2018).

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## 11 Clinical Trials

MSC derived from different sources such as bone marrow umbilical cord and adipose tissue have been used in clinical trials. Although MSC did not display curative properties it is quite astonishing that a partial improvement of glycemia has been reported (Päth et al. 2019). To date [ClinicalTrials.gov](https://clinicaltrials.gov) listed a number of therapeutic approaches using different cell types. Among them over 850 have used MSC to target a broad variety of diseases and 60 of them for T1DM and T2DM. A trial investigating the islet graft survival and function of MSC co-transplantation has been closed (Päth et al. 2019). It has been demonstrated that MSC mediate immune tolerance enabling a partial recovery of residual  $\beta$ -cell mass and/or delaying the  $\beta$ -cell damage during the onset of T1DM (Päth et al. 2019). Some of these clinical trials using co-transplanted MSC were found to reduce the loss of islet graft, the HbA1c percentage and to increase the release of C-peptide compared to control patients during 2 years follow-up (Päth et al. 2019). However, currently the small number of patients recruited for the studies dictate the requirement of a more prolonged observational study to elucidate whether MSC could really delay the development of T1DM (Päth et al. 2019). Furthermore different strategies using ESC iPSC and MSCs are still under investigation (Welsch et al. 2018). Moreover, since cellular debris and fragments of proteins may be sufficient to prime the immune system using the encapsulation devices an effective encapsulation device should be generated to prevent activation of the immune response leading to graft rejection. It has been shown that Encaptra devices are able to normalize blood glucose in spontaneous NOD



**Fig. 2 Generation of POs from HLSC.** (a) Non-differentiated HLSC showing their typical morphology. (b) Generation of HLSC-derived islet-like structures. (c) HLSC-ILS stained with fluorescein diacetate (FDA) and propidium iodide (PI) show viable (green) and dead cells (red, arrow). (d) Immunofluorescence for human

C-peptide (red) and Glucose transporter 2 (GLUT, green) in HLSC-ILS. (e) Transmission electron microscopy showing typical  $\beta$ -cell granules. (f) Glucose-stimulated C-peptide secretion in 2.8 mM glucose (LG) and 28 mM glucose (HG)

recipients (Faleo et al. 2016). In addition, in 2014 ViaCyte has started a phase I/II clinical trial ([ClinicalTrials.gov](http://ClinicalTrials.gov) identifier: Nbib2239354) currently ongoing. In 2017 a new Phase 1/2 clinical trial was started (STEP ONE) to test the PEC-Direct™ in patients with T1DM in San Diego (California USA) and Edmonton (Alberta Canada). More recently, the clinical trial was extended to Baltimore (Maryland USA) Minneapolis (Minnesota USA) Columbus (Ohio USA) and Vancouver (British Columbia Canada) to test the safety and efficacy of the ViaCyte's PEC-Encap (a.k.a. VC-01™) ([ClinicalTrials.gov](http://ClinicalTrials.gov) identifier: NCT03163511). The innovation consists in the use of a new open device that allows a direct vascularization of pancreatic progenitor cells (PEC-01) and avoids the use of immunosuppressive drugs. These studies are still ongoing but they could provide important information on safety and effectiveness for the use of stem cell-derived  $\beta$ -cells in humans. ([Clinicaltrials.gov](http://Clinicaltrials.gov).)

The failure to generate feasible implantable devices to be applied in diabetic setting is still a challenge for many companies. This mainly depends on the fibrotic processes, the oxygenation and the diffusion of nutrients allowing a long-term PO viability.

## 12 Conclusions

In the last decades, the generation of insulin-secreting cells has gained particular attention. Different cell sources have been used and a number of studies have shown that functional insulin-secreting cells can be generated (Zhou and Melton 2018; Matsumoto et al. 2016; Ravassard et al. 2011; Boss et al. 2017; Clark et al. 1997; Scharfmann et al. 2014; Xie et al. 2016; Balboa et al. 2018; Wang et al. 2017; Russ et al. 2008; Avnit-Sagi et al. 2009; Russ et al. 2011; Russ et al. 2009; Joglekar et al. 2009; Klein et al. 2013;

Latreille et al. 2015; Carlotti et al. 2010; Zulewski et al. 2001; Gallo et al. 2007). Nevertheless, their clinical application is still missing.

Being organoids versatile for applications they have gained interest. In particular, the 3D conformation plays an important role in morphogenesis and tissue organization of pancreatic islets (Navarro-tableros et al. 2018; Kim et al. 2016a). Cell assembly in 3D structures facilitates cellular interactions, promotes morphogenesis and tissue organization, but also a fine modulation of hormone secretion (Orci et al. 1975; Kitasato et al. 1996; Kanno et al. 2002; Wojtuszczyński et al. 2008; Jo et al. 2009; Klee et al. 2011; Santos-Silva et al. 2012; Rorsman and Ashcroft 2018). Thereby, generation of POs, recapitulating the structure and function of native pancreatic islets, should be evaluated for long-term transplant viability. Moreover, organoid cultures are ideal to study stem cell-niche interactions in a 3D environment, and engineering POs could serve for drug studies (Scavuzzo et al. 2018).

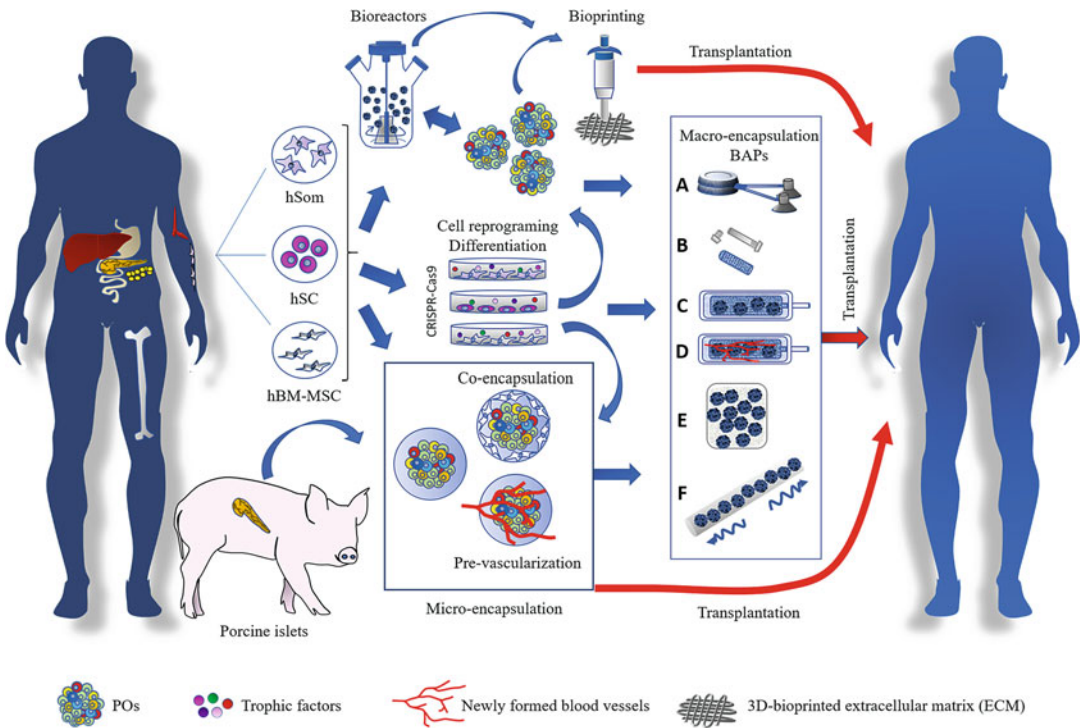
Ideally, optimization would include feasible cell sources for a large production of functional POs. Thereby, generation of POs, mimicking the human native pancreatic islets in terms of morphology and function, is mandatory. Staminal cells isolated from a number of embryonic and adult tissues are currently used to generate functional POs (Zhou and Melton 2018; Balboa et al. 2018; Navarro-tableros et al. 2018; Kim et al. 2016a; Hinderer et al. 2016; Liu et al. 2018) (Fig. 3). Nevertheless, standardization of protocols for POs generation is still needed to achieve reproducibility and large scalability. Furthermore, efforts should be directed to generate mature POs, similar to native pancreatic islets, in self-regulation of glucose metabolism. The recent gene editing technology is a challenge to generate POs from different cell types and to educate cells obtained from T1DM patients in order to generate functional POs for autologous transplantation (Giménez et al. 2016; Ma et al. 2018).

The 3D bioreactor-based culture systems have demonstrated to improve the production of functional stem cell-derived  $\beta$ -like cells *in vitro* (Abu-Absi et al. 2014; Hoesli et al. 2009; Sharp and Vermette 2017; Gundersen et al. 2010)

(Fig. 3). Additionally, functional POs generated *in vitro*, were able to secrete insulin in response to glucose, and to restore the glycemic control when implanted in diabetic animals (Vaithilingam et al. 2011; Mason et al. 2009; Amer et al. 2015; Lee et al. 2006a; Jiang et al. 2017; An et al. 2018; Ludwig et al. 2012; Montazeri et al. 2016; Mahou et al. 2017; Faleo et al. 2016). Nevertheless, similarly to transplanted human pancreatic islets, oxygenation and activation of the host immune response are the main remaining hurdles.

To solve this problem, bioengineers and nanotechnologists have generated a number of new promising encapsulation approaches (Gálvez-Martín et al. 2017). Currently, nanotechnology research is focused on the generation of POs with a 3D morphology by using different polymers and natural ECM as encapsulating platforms (Gálvez-Martín et al. 2017) (Fig. 3). Pre-clinical studies have demonstrated their potential application to enhance PO vascularization (De Rosa et al. 2018; Schweicher et al. 2014; Trivedi et al. 2000; Sweet et al. 2008; Mahou et al. 2017; Guo et al. 2018; Skrzypek et al. 2018a, b; Groot Nibbelink et al. 2018; Stephens et al. 2018) and to improve the immune escape (Hinderer et al. 2016; Amer et al. 2015; Rios et al. 2016; Buitinga et al. 2013; Lee et al. 2004, 2006a, b, c, 2009; Jiang et al. 2017; Bowers et al. 2018; Liao et al. 2013; Andrades et al. 2008; Lou et al. 2017; Hamilton et al. 2017; Chaimov et al. 2017). Nevertheless, different clinical trials, many of which still ongoing, were not conclusive in confirming their safety for clinical application (ClinicalTrials.gov).

A number of pre-clinical cell-based approaches have been also developed and each approach has contributed to solve the most relevant challenges. Indeed, the development of pre-vascularized or oxygenated POs represent the more promising and powerful tools for translational applications (Fig. 3). Finally, even though different clinical trials are currently ongoing to evaluate the potential application in diabetes, standardization of protocols for PO generation are still required to achieve reproducibility. Improvements should also include the feasibility and safety as well as the tuning of suitable protocols to obtain fully mature POs.



**Fig. 3** Current strategies to generate 3D pancreatic organoids (POs)

A number of human cells including somatic (hSom), stem cells (hSC) and bone marrow-mesenchymal stem cells (hBM-MSC) as well as cells obtained from different embryonic or adult organs are currently used for their potential to generate *in vitro*, functional POs

*In vitro* cell commitment is promoted by the addition of specific trophic factors or by gene editing (CRISPR-Cas9). Encapsulation allows oxygen, nutrients to pass through the porous membrane. Encapsulation also offers immunoprotection, promotes survival, differentiation, maturation and glucose-responsive insulin secretion. POs generated from human cells or from porcine islets (xenografts) can be macro-encapsulated and/or alginate-based micro-encapsulated to improve long-term survival.

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Pre-vascularization or addition of oxygen generators are two potential approaches which could solve the reduced oxygen supply of transplanted POs

Bioartificial pancreas systems (BAPs) have been proposed to avoid the host immune attack against POs. The figure shows a schematic representation of some commercial BAP. βAir® Chamber System (a), Semnova’s Cell Pouch System™ schematization (b), TheraCyte™ system schematization (c), VC-01TM (Pro-vascularized) System (d), Islets Sheet System (e) and Silk Fibroinc System (f). 3D bioprinting modalities are also used to generate ECM-containing POs. Natural or synthetic extracellular matrix (nECM, sECM) components are used for this purpose.

Bioreactors are proposed to large-scalability PO production.

**References**

Aamodt KI, Powers AC (2017) Signals in the pancreatic islet microenvironment influence β-cell proliferation. *Diabetes Obes Metab.* <https://doi.org/10.1111/dom.13031>

Abazari MF, Soleimanifar F, Nouri Aleagha M, Torabinejad S, Nasiri N, Khamisipour G, Amini Mahabadi J, Mahboudi H, Enderami SE, Saburi E, Hashemi J, Kehtari M (2018) PCL/PVA nanofibrous scaffold improve insulin-producing cells generation from human induced pluripotent stem cells. *Gene.* <https://doi.org/10.1016/j.gene.2018.05.115>

- Abu-Absi S, Xu S, Graham H, Dalal N, Boyer M, Dave K (2014) Cell culture process operations for recombinant protein production. *Adv Biochem Eng Biotechnol*. [https://doi.org/10.1007/10\\_2013\\_252](https://doi.org/10.1007/10_2013_252)
- Afelik S, Rovira M (2017) Pancreatic  $\beta$ -cell regeneration: facultative or dedicated progenitors? *Mol Cell Endocrinol*. <https://doi.org/10.1016/j.mce.2016.11.008>
- Affeld K, Schaller J, Wölken T, Krabatsch T, Kertzscher U (n.d.) Role of flow for the deposition of platelets. *Biointerphases*. <https://doi.org/10.1116/1.4944383>. Diventa 208 en la forma correctA
- Agulnick AD, Ambruzs DM, Moorman MA, Bhounik A, Cesario RM, Payne JK, Kelly JR, Haakmeester C, Srijemac R, Wilson AZ, Kerr J, Frazier MA, Kroon EJ, D'Amour KA (2015) Insulin-producing endocrine cells differentiated in vitro from human embryonic stem cells function in macroencapsulation devices in vivo. *Stem Cells Transl Med*. <https://doi.org/10.5966/sctm.2015-0079>
- Alismaïl H, Jin S (2014) Microenvironmental stimuli for proliferation of functional islet  $\beta$ -cells. *Cell Biosci*. <https://doi.org/10.1186/2045-3701-4-12>
- Amer LD, Holtzinger A, Keller G, Mahoney MJ, Bryant SJ (2015) Enzymatically degradable poly(ethylene glycol) hydrogels for the 3D culture and release of human embryonic stem cell derived pancreatic precursor cell aggregates. *Acta Biomater*. <https://doi.org/10.1016/j.actbio.2015.04.013>
- An D, Chiu A, Flanders JA, Song W, Shou D, Lu Y-C, Grunnet LG, Winkel L, Ingvorsen C, Christophersen NS, Fels JJ, Sand FW, Ji Y, Qi L, Pardo Y, Luo D, Silberstein M, Fan J, Ma M (2018) Designing a retrievable and scalable cell encapsulation device for potential treatment of type 1 diabetes. *Proc Natl Acad Sci*. <https://doi.org/10.1073/pnas.1708806115>
- Andrades P, Asiedu CK, Gansuud B, Inusah S, Goodwin KJ, Deckard LA, Jargal U, Thomas JM (2008) Pancreatic islet isolation variables in non-human primates (rhesus macaques). *Diabetologia*. <https://doi.org/10.1007/s00125-008-1030-z>
- Anitua E, Nurden P, Prado R, Nurden AT, Padilla S (2019) Autologous fibrin scaffolds: when platelet- and plasma-derived biomolecules meet fibrin. *Biomaterials*. <https://doi.org/10.1016/j.biomaterials.2018.11.029>
- Avnit-Sagi T, Kantorovich L, Kredon-Russo S, Hornstein E, Walker MD (2009) The promoter of the pri-miR-375 gene directs expression selectively to the endocrine pancreas. *PLoS One*. <https://doi.org/10.1371/journal.pone.0005033>
- Azadi SA, Vashghani-Farahani E, Hashemi-Najafabadi-S, Godini A (2016) Co-encapsulation of pancreatic islets and pentoxifylline in alginate-based microcapsules with enhanced immunosuppressive effects. *Prog Biomater*. <https://doi.org/10.1007/s40204-016-0049-3>
- Badylak SF, Taylor D, Uygun K, Sabetkish S, Kajbafzadeh AM, Sabetkish N, Khorramirouz R, Akbarzadeh A, Seyedian SL, Pasalar P, Orangian S, Beigi RSH, Aryan Z, Akbari H, Tavangar SM (2011) Whole-organ tissue engineering: decellularization and recellularization of three-dimensional matrix scaffolds. *Annu Rev Biomed Eng*. <https://doi.org/10.1146/annurev-bioeng-071910-124743>
- Balboa D, Saarimäki-vire J, Otonkoski T (2018) Human pluripotent stem cells for the modelling of pancreatic beta-cell pathology. *Stem Cells*. <https://doi.org/10.1002/stem.2913>
- Barati G, Nadri S, Hajian R, Rahmani A, Mostafavi H, Mortazavi Y, Taromchi AH (2018) Differentiation of microfluidic-encapsulated trabecular meshwork mesenchymal stem cells into insulin producing cells and their impact on diabetic rats. *J Cell Physiol*. <https://doi.org/10.1002/jcp.27426>
- Barkai U, Weir GC, Colton CK, Ludwig B, Bornstein SR, Brendel MD, Neufeld T, Bremer C, Leon A, Evron Y, Yavriyants K, Azarov D, Zimmermann B, Maimon S, Shabtay N, Balyura M, Rozenshtein T, Vardi P, Bloch K, De Vos P, Rotem A (2013) Enhanced oxygen supply improves islet viability in a new bioartificial pancreas. *Cell Transplant*. <https://doi.org/10.3727/096368912X657341>
- Bellin MD, Barton FB, Heitman A, Harmon JV, Kandaswamy R, Balamurugan AN, Sutherland DER, Alejandro R, Hering BJ (2012) Potent induction immunotherapy promotes long-term insulin independence after islet transplantation in type 1 diabetes. *Am J Transplant*. <https://doi.org/10.1111/j.1600-6143.2011.03977.x>
- Bergert H, Knoch KP, Meisterfeld R, Jäger M, Ouwendijk J, Kersting S, Saeger HD, Solimena M (2005) Effect of oxygenated perfluorocarbons on isolated rat pancreatic islets in culture. *Cell Transplant*. <https://doi.org/10.3727/000000005783982873>
- Berman DM, Molano RD, Fotino C, Ulissi U, Gimeno J, Mendez AJ, Kenyon NM, Kenyon NS, Andrews DM, Ricordi C, Pileggi A (2016) Bioengineering the endocrine pancreas: intra-organ islet transplantation within a biologic resorbable scaffold. *Diabetes*. <https://doi.org/10.2337/db15-1525>
- Bettinger CJ, Langer R, Borenstein JT (2009) Engineering substrate topography at the micro- and nanoscale to control cell function. *Angew Chem Int Ed Engl*. <https://doi.org/10.1002/anie.200805179>
- Blomeier H, Zhang X, Rives C, Brissova M, Hughes E, Baker M, Powers AC, Kaufman DB, Shea LD, Lowe WL (2006) Polymer scaffolds as synthetic microenvironments for extrahepatic islet transplantation. *Transplantation*. <https://doi.org/10.1097/01.tp.0000231708.19937.21>
- Blyszczuk P, Czyz J, Kania G, Wagner M, Roll U, St-Onge L, Wobus AM (2003) Expression of Pax4 in embryonic stem cells promotes differentiation of nestin-positive progenitor and insulin-producing cells. *Proc Natl Acad Sci U S A*. <https://doi.org/10.1073/pnas.0237371100>
- Boettler T, Schneider D, Cheng Y, Kadoya K, Brandon EP, Martinson L, Von Herrath M (2016) Pancreatic tissue transplanted in TheraCyte™ encapsulation devices is

- protected and prevents hyperglycemia in a mouse model of immune-mediated diabetes. *Cell Transplant*. <https://doi.org/10.3727/096368915X688939>
- Boss C, De Marchi U, Hermant A, Conrad M, Sizzano F, Palini A, Wiederkehr A, Bouche N (2017) Encapsulation of insulin-secreting cells expressing a genetically encoded fluorescent calcium indicator for cell-based sensing in vivo. *Adv Healthc Mater*. <https://doi.org/10.1002/adhm.201600869>
- Bowers DT, Olingy CE, Chhabra P, Langman L, Merrill PH, Linhart RS, Tanes ML, Lin D, Brayman KL, Botchwey EA (2018) An engineered macroencapsulation membrane releasing FTY720 to precondition pancreatic islet transplantation. *J Biomed Mater Res B Appl Biomater*. <https://doi.org/10.1002/jbm.b.33862>
- Breger JC, Fisher B, Samy R, Pollack S, Wang NS, Isayeva I (2015) Synthesis of “click” alginate hydrogel capsules and comparison of their stability, water swelling, and diffusion properties with that of Ca<sup>2+</sup> crosslinked alginate capsules. *J Biomed Mater Res B Appl Biomater*. <https://doi.org/10.1002/jbm.b.33282>
- Brennan DC, Kopetskie HA, Sayre PH, Alejandro R, Cagliero E, Shapiro AMJ, Goldstein JS, DesMarais MR, Booher S, Bianchine PJ (2016) Long-term follow-up of the Edmonton protocol of islet transplantation in the United States. *Am J Transplant*. <https://doi.org/10.1111/ajt.13458>
- Brennand K, Huangfu D, Melton D (2007) All  $\beta$  cells contribute equally to islet growth and maintenance. *PLoS Biol*. <https://doi.org/10.1371/journal.pbio.0050163>
- Brown BN, Badylak SF (2015) Extracellular matrix as an inductive scaffold for functional tissue reconstruction. *Transl Res*. <https://doi.org/10.1016/j.trsl.2013.11.003>
- Bruni A, Gala-Lopez B, Pepper AR, Abualhassan NS, James Shapiro AM (2014) Islet cell transplantation for the treatment of type 1 diabetes: recent advances and future challenges. *Diabetes Metab Syndr Obes Targets Ther*. <https://doi.org/10.2147/DMSO.S50789>
- Buchwald P, Tamayo-Garcia A, Manzoli V, Tomei AA, Stabler CL (2018) Glucose-stimulated insulin release: parallel perfusion studies of free and hydrogel encapsulated human pancreatic islets. *Biotechnol Bioeng*. <https://doi.org/10.1002/bit.26442>
- Buitinga M, Truckenmüller R, Engelse MA, Moroni L, Ten Hoopen HWM, van Blitterswijk CA, de Koning EJP, van Apeldoorn AA, Karperien M (2013) Microwell scaffolds for the Extrahepatic transplantation of islets of Langerhans. *PLoS One*. <https://doi.org/10.1371/journal.pone.0064772>
- Buitinga M, Assen F, Hanegraaf M, Wieringa P, Hilderink J, Moroni L, Truckenmüller R, van Blitterswijk C, Römer GW, Carlotti F, de Koning E, Karperien M, van Apeldoorn A (2017) Microfabricated scaffolds lead to efficient remission of diabetes in mice. *Biomaterials*. <https://doi.org/10.1016/j.biomaterials.2017.03.031>
- Cabric S, Sanchez J, Lundgren T, Foss A, Felldin M, Källén R, Salmela K, Tibell A, Tufveson G, Larsson R, Korsgren O, Nilsson B (2007) Islet surface heparinization prevents the instant blood-mediated inflammatory reaction in islet transplantation. *Diabetes*. <https://doi.org/10.2337/db07-0358>
- Cabric S, Sanchez J, Johansson U, Larsson R, Nilsson B, Korsgren O, Magnusson PU (2010) Anchoring of vascular endothelial growth factor to surface-immobilized heparin on pancreatic islets: implications for stimulating islet angiogenesis. *Tissue Eng Part A*. <https://doi.org/10.1089/ten.tea.2009.0429>
- Calafiore R, Basta G, Luca G, Lemmi A, Montanucci MP, Calabrese G, Racanicchi L, Mancuso F, Brunetti P (2006) Microencapsulated pancreatic islet allografts into nonimmunosuppressed patients with type 1 diabetes. *Diabetes Care*. <https://doi.org/10.2337/diacare.29.01.06.dc05-1270>
- Cameron D, Hushen J, Nazian S (2001) Formation of insulin-secreting, Sertoli-enriched tissue constructs by microgravity coculture of isolated pig islets and rat sertoli cells. *In Vitro Cell Dev Biol Anim* 37:490–498
- Cardona K, Korbutt GS, Milas Z, Lyon J, Cano J, Jiang W, Bello-Laborn H, Hacquoil B, Strobert E, Gangappa S, Weber CJ, Pearson TC, Rajotte RV, Larsen CP (2006) Long-term survival of neonatal porcine islets in non-human primates by targeting costimulation pathways. *Nat Med*. <https://doi.org/10.1038/nm1375>
- Carlotti F, Zaldumbide A, Loomans CJ, van Rossenberg E, Engelse M, de Koning EJP, Hoeben RC (2010) Isolated human islets contain a distinct population of mesenchymal stem cells. *Islets*. <https://doi.org/10.4161/isl.2.3.11449>
- Carlsson PO, Palm F, Andersson A, Liss P (2001) Markedly decreased oxygen tension in transplanted rat pancreatic islets irrespective of the implantation site. *Diabetes*. <https://doi.org/10.2337/diabetes.50.3.489>
- Carlsson PO, Espes D, Sedigh A, Rotem A, Zimmerman B, Grinberg H, Goldman T, Barkai U, Avni Y, Westermark GT, Carlborn L, Ahlström H, Eriksson O, Olerud J, Korsgren O (2018) Transplantation of macroencapsulated human islets within the bioartificial pancreas  $\beta$ Air to patients with type 1 diabetes mellitus. *Am J Transplant*. <https://doi.org/10.1111/ajt.14642>
- Celikkin N, Rinoldi C, Costantini M, Trombetta M, Rainer A, Świążkowski W (2017) Naturally derived proteins and glycosaminoglycan scaffolds for tissue engineering applications. *Mater Sci Eng C Mater Biol Appl*. <https://doi.org/10.1016/j.msec.2017.04.016>
- Chaimov D, Baruch L, Krishdul S, Meivar-levy I, Ferber S, Machluf M (2017) Innovative encapsulation platform based on pancreatic extracellular matrix achieve substantial insulin delivery. *J Control Release*. <https://doi.org/10.1016/j.jconrel.2016.07.045>
- Chawla M, Bodnar CA, Sen A, Kallos MS, Behie LA (2006) Production of islet-like structures from neonatal porcine pancreatic tissue in suspension bioreactors. *Biotechnol Prog*. <https://doi.org/10.1021/bp050261i>
- Chawla K, Bin YT, Liao SW, Guan Z (2011) Biodegradable and biocompatible synthetic saccharide-peptide

- hydrogels for three-dimensional stem cell culture. *Biomacromolecules*. <https://doi.org/10.1021/bm100980w>
- Chayosumrit M, Tuch B, Sidhu K (2010) Alginate microcapsule for propagation and directed differentiation of hESCs to definitive endoderm. *Biomaterials*. <https://doi.org/10.1016/j.biomaterials.2009.09.071>
- Chen T, Yuan J, Duncanson S, Hibert ML, Kodish BC, Mylavaganam G, Maker M, Li H, Sremac M, Santosuosso M, Forbes B, Kashiwagi S, Cao J, Lei J, Thomas M, Hartono C, Sachs D, Markmann J, Sambanis A, Poznansky MC (2015) Alginate encapsulant incorporating CXCL12 supports long-term allo- and xenoislet transplantation without systemic immune suppression. *Am J Transplant*. <https://doi.org/10.1111/ajt.13049>
- Cheng JYC, Raghunath M, Whitelock J, Poole-Warren L (2011) Matrix components and scaffolds for sustained islet function. *Tissue Eng Part B Rev*. <https://doi.org/10.1089/ten.teb.2011.0004>
- Clark SA, Quaade C, Constandy H, Hansen P, Halban P, Ferber S, Newgard CB, Normington K (1997) Novel insulinoma cell lines produced by iterative engineering of GLUT2, glucokinase, and human insulin expression. *Diabetes*. <https://doi.org/10.2337/diab.46.6.958>
- Croon AC, Karlsson R, Bergström C, Björklund E, Möller C, Tydén L, Tibell A (2003) Lack of donors limits the use of islet transplantation as treatment for diabetes. *Transplant Proc* 35(2):764
- Crossan C, Mourad NI, Smith K, Gianello P, Scobie L (2018) Assessment of porcine endogenous retrovirus transmission across an alginate barrier used for the encapsulation of porcine islets. *Xenotransplantation*. <https://doi.org/10.1111/xen.12409>
- Daoud J, Rosenberg L, Tabrizian M (2010) Pancreatic islet culture and preservation strategies: advances, challenges, and future outlook. *Cell Transplant*. <https://doi.org/10.3727/096368910X515872>
- Datar A, Joshi P, Lee MY (2015) Biocompatible hydrogels for microarray cell printing and encapsulation. *Biosensors (Basel)*. <https://doi.org/10.3390/bios5040647>
- Davani B, Ikononou L, Raaka BM, Geras-Raaka E, Morton RA, Marcus-Samuels B, Gershengorn MC (2007) Human islet-derived precursor cells are mesenchymal stromal cells that differentiate and mature to hormone-expressing cells in vivo. *Stem Cells*. <https://doi.org/10.1634/stemcells.2007-0323>
- Davis NE, Beenken-Rothkopf LN, Mirsoian A, Kojic N, Kaplan DL, Barron AE, Fontaine MJ (2012) Enhanced function of pancreatic islets co-encapsulated with ECM proteins and mesenchymal stromal cells in a silk hydrogel. *Biomaterials*. <https://doi.org/10.1016/j.biomaterials.2012.06.015>
- De Mesmaeker I, Robert T, Suenens KG, Stangé GM, Van Hulle F, Ling Z, Tomme P, Jacobs-Tulleneers-Thevissen D, Keymeulen B, Pipeleers DG (2018) Increase functional  $\beta$ -cell mass in subcutaneous alginate capsules with porcine prenatal islet cells but loss with human adult islet cells. *Diabetes*. <https://doi.org/10.2337/db18-0709>
- De Rosa L, Di Stasi R, D'Andrea LD (2018) Pro-angiogenic peptides in biomedicine. *Arch Biochem Biophys*. <https://doi.org/10.1016/j.abb.2018.10.010>
- de Vos P, Faas MM, Strand B, Calafiore R (2006) Alginate-based microcapsules for immunoisolation of pancreatic islets. *Biomaterials* 27(32):5603–5617
- De Vos P, Lazarjani HA, Poncelet D, Faas MM (2014) Polymers in cell encapsulation from an enveloped cell perspective. *Adv Drug Deliv Rev*. <https://doi.org/10.1016/j.addr.2013.11.005>
- De Vos P, Smink AM, Paredes G, Lakey JRT, Kuipers J, Giepmans BNG, De Haan BJ, Faas MM (2016) Enzymes for pancreatic islet isolation impact chemokine-production and polarization of insulin-producing  $\beta$ -cells with reduced functional survival of immunoisolated rat islet-allografts as a consequence. *PLoS One*. <https://doi.org/10.1371/journal.pone.0147992>
- Desai T, Shea LD (2017) Advances in islet encapsulation technologies. *Nat Rev Drug Discov*. <https://doi.org/10.1038/nrd.2016.232>
- Desai NM, Goss JA, Deng S, Wolf BA, Markmann E, Palanjian M, Shock AP, Feliciano S, Brunicaardi FC, Barker CF, Naji A, Markmann JF (2003) Elevated portal vein drug levels of sirolimus and tacrolimus in islet transplant recipients: local immunosuppression or islet toxicity. *Transplantation* 76:1623–1625. <https://doi.org/10.1097/01.TP.0000081043.23751.81>
- Dimatteo R, Darling NJ, Segura T (2018) In situ forming injectable hydrogels for drug delivery and wound repair. *Adv Drug Deliv Rev*. <https://doi.org/10.1016/j.addr.2018.03.007>
- Dolgin E (2016) Diabetes: encapsulating the problem. *Nature*. <https://doi.org/10.1038/540S60a>
- Donadel G, Pastore D, Della-Morte D, Capuani B, Lombardo MF, Pacifici F, Bugliani M, Grieco FA, Marchetti P, Lauro D (2017) FGF-2b and h-PL transform duct and non-endocrine human pancreatic cells into endocrine insulin secreting cells by modulating differentiating genes. *Int J Mol Sci*. <https://doi.org/10.3390/ijms18112234>
- Dor Y, Brown J, Martinez OI, Melton DA (2004) Adult pancreatic  $\beta$ -cells are formed by self-duplication rather than stem-cell differentiation. *Nature*. <https://doi.org/10.1038/nature02520>
- Dufrane D, Goebbels RM, Saliez A, Guiot Y, Gianello P (2006) Six-month survival of microencapsulated pig islets and alginate biocompatibility in primates: proof of concept. *Transplantation*. <https://doi.org/10.1097/01.tp.0000208610.75997.20>
- Dufrane D, Goebbels RM, Gianello P (2010) Alginate macroencapsulation of pig islets allows correction of streptozotocin-induced diabetes in primates up to 6 months without immunosuppression. *Transplantation*. <https://doi.org/10.1097/TP.0b013e3181f6e267>

- Elliott RB, Escobar L, Tan PLJ, Garkavenko O, Calafiore R, Basta P, Vasconcellos AV, Emerich DF, Thanos C, Bambra C (2005) Intraperitoneal alginate-encapsulated neonatal porcine islets in a placebo-controlled study with 16 diabetic cynomolgus primates. *Transplant Proc* 37(8):3505–3508
- Ellis C, Ramzy A, Kieffer TJ (2017) Regenerative medicine and cell-based approaches to restore pancreatic function. *Nat Rev Gastroenterol Hepatol*. <https://doi.org/10.1038/nrgastro.2017.93>
- Enderami SE, Mortazavi Y, Soleimani M, Nadri S, Biglari A, Mansour RN (2017) Generation of insulin-producing cells from human-induced pluripotent stem cells using a stepwise differentiation protocol optimized with platelet-rich plasma. *J Cell Physiol*. <https://doi.org/10.1002/jcp.25721>
- Evron Y, Zimmermann B, Ludwig B, Barkai U, Colton CK, Weir GC, Arieli B, Maimon S, Shalev N, Yavriyants K, Goldman T, Gendler Z, Eizen L, Vardi P, Bloch K, Barthel A, Bornstein SR, Rotem A (2015) Oxygen supply by photosynthesis to an implantable islet cell device. *Horm Metab Res*. <https://doi.org/10.1055/s-0034-1394375>
- Faleo G, Lee K, Nguyen V, Tang Q (2016) Assessment of immune isolation of allogeneic mouse pancreatic progenitor cells by a macroencapsulation device. *Transplantation* 100:1211–1218. <https://doi.org/10.1097/TP.0000000000001146>
- Faleo G, Russ HA, Wisel S, Parent AV, Nguyen V, Nair GG, Freise JE, Villanueva KE, Szot GL, Hebrok M, Tang Q (2017) Mitigating ischemic injury of stem cell-derived insulin-producing cells after transplant. *Stem Cell Rep*. <https://doi.org/10.1016/j.stemcr.2017.07.012>
- Farina M, Ballerini A, Fraga DW, Nicolov E, Hogan M, Demarchi D, Scaglione F, Sabek OM, Horner P, Thekkedath U, Gaber OA, Grattoni A (2017) 3D printed vascularized device for subcutaneous transplantation of human islets. *Biotechnol J*. <https://doi.org/10.1002/biot.201700169>
- Farina M, Chua CYX, Ballerini A, Thekkedath U, Alexander JF, Rhudy JR, Torchio G, Fraga D, Pathak RR, Villanueva M, Shin CS, Niles JA, Sesana R, Demarchi D, Sikora AG, Acharya GS, Gaber AO, Nichols JE, Grattoni A (2018) Transcutaneously refillable, 3D-printed biopolymeric encapsulation system for the transplantation of endocrine cells. *Biomaterials*. <https://doi.org/10.1016/j.biomaterials.2018.05.047>
- Formo K, Cho CHH, Vallier L, Strand BL (2015) Culture of hESC-derived pancreatic progenitors in alginate-based scaffolds. *J Biomed Mater Res A*. <https://doi.org/10.1002/jbm.a.35507>
- Foster JL, Williams G, Williams LJ, Tuch BE (2007) Differentiation of transplanted microencapsulated fetal pancreatic cells. *Transplantation*. <https://doi.org/10.1097/01.tp.0000264555.46417.7d>
- Gallo R, Gambelli F, Gava B, Sasdelli F, Tellone V, Masini M, Marchetti P, Dotta F, Sorrentino V (2007) Generation and expansion of multipotent mesenchymal progenitor cells from cultured human pancreatic islets. *Cell Death Differ*. <https://doi.org/10.1038/sj.cdd.4402199>
- Gálvez-Martín P, Martín JM, Ruiz AM, Clares B (2017) Encapsulation in cell therapy: methodologies, materials, and clinical applications. *Curr Pharm Biotechnol*. <https://doi.org/10.2174/1389201018666170502113252>
- Gerace D, Martiniello-Wilks R, Nassif NT, Lal S, Steptoe R, Simpson AM (2017) CRISPR-targeted genome editing of mesenchymal stem cell-derived therapies for type 1 diabetes: a path to clinical success? *Stem Cell Res Ther*. <https://doi.org/10.1186/s13287-017-0511-8>
- Giménez CA, Ielpi M, Mutto A, Grosebacher L, Argibay P, Pereyra-Bonnet F (2016) CRISPR-on system for the activation of the endogenous human INS gene. *Gene Ther*. <https://doi.org/10.1038/gt.2016.28>
- Goh F, Gross JD, Simpson NE, Sambanis A (2010) Limited beneficial effects of perfluorocarbon emulsions on encapsulated cells in culture: experimental and modeling studies. *J Biotechnol*. <https://doi.org/10.1016/j.jbiotec.2010.08.013>
- Goh SK, Bertera S, Olsen P, Candiello JE, Halfter W, Uechi G, Balasubramani M, Johnson SA, Sicari BM, Kollar E, Badylak SF, Banerjee I (2013) Perfusion-decellularized pancreas as a natural 3D scaffold for pancreatic tissue and whole organ engineering. *Biomaterials*. <https://doi.org/10.1016/j.biomaterials.2013.05.066>
- Groot Nibbelink M, Skrzypek K, Karbaat L, Both S, Plass J, Klomphaar B, van Lente J, Henke S, Karperien M, Stamatialis D, van Apeldoorn A (2018) An important step towards a prevascularized islet microencapsulation device: in vivo prevascularization by combination of mesenchymal stem cells on micropatterned membranes. *J Mater Sci Mater Med*. <https://doi.org/10.1007/s10856-018-6178-6>
- Guilak F, Cohen DM, Estes BT, Gimble JM, Liedtke W, Chen CS (2009) Control of stem cell fate by physical interactions with the extracellular matrix. *Cell Stem Cell*. <https://doi.org/10.1016/j.stem.2009.06.016>
- Gundersen SI, Chen G, Powell HM, Palmer AF (2010) Hemoglobin regulates the metabolic and synthetic function of rat insulinoma cells cultured in a hollow fiber bioreactor. *Biotechnol Bioeng*. <https://doi.org/10.1002/bit.22830>
- Guo Y, Wu C, Xu L, Xu Y, Xiaohong L, Hui Z, Jingjing L, Lu Y, Wang Z (2018) Vascularization of pancreatic decellularized scaffold with endothelial progenitor cells. *J Artif Organs*. <https://doi.org/10.1007/s10047-018-1017-6>
- Guruswamy Damodaran R, Vermette P (2018a) Tissue and organ decellularization in regenerative medicine. *Biotechnol Prog*. <https://doi.org/10.1002/btpr.2699>
- Guruswamy Damodaran R, Vermette P (2018b) Decellularized pancreas as a native extracellular matrix scaffold for pancreatic islet seeding and culture. *J Tissue Eng Regen Med*. <https://doi.org/10.1002/term.2655>



- Hamilton DC, Shih HH, Schubert RA, Michie SA, Staats PN, Kaplan DL, Fontaine MJ (2017) A silk-based encapsulation platform for pancreatic islet transplantation improves islet function in vivo. *J Tissue Eng Regen Med.* <https://doi.org/10.1002/term.1990>
- Haque MR, Kim J, Park H, Lee HS, Lee KW, Al-Hilal TA, Jeong JH, Ahn CH, Lee DS, Kim SJ, Byun Y (2017) Xenotransplantation of layer-by-layer encapsulated non-human primate islets with a specified immunosuppressive drug protocol. *J Control Release.* <https://doi.org/10.1016/j.jconrel.2017.04.021>
- Hashemi J, Pasalar P, Soleimani M, Arefian E, Khorramirouz R, Akbarzadeh A, Ghorbani F, Enderami SE, Kajbafzadeh AM (2018) Decellularized pancreas matrix scaffolds for tissue engineering using ductal or arterial catheterization. *Cells Tissues Organs.* <https://doi.org/10.1159/000487230>
- He L, Ahmad M, Perrimon N (2018) Mechanosensitive channels and their functions in stem cell differentiation. *Exp Cell Res.* S0014482718311194
- Henriksen J, Lau J, Zang G, Berggren PO, Köhler M, Carlsson PO (2012) Markedly decreased blood perfusion of pancreatic islets transplanted intraportally into the liver: disruption of islet integrity necessary for islet revascularization. *Diabetes.* <https://doi.org/10.2337/db10-0895>
- Hering BJ, Wijkstrom M, Graham ML, Hårdstedt M, Aasheim TC, Jie T, Ansit JD, Nakano M, Cheng J, Li W, Moran K, Christians U, Finnegan C, Mills CD, Sutherland DE, Bansal-Pakala P, Murtaugh MP, Kirchoff N, Schuurman HJ (2006) Prolonged diabetes reversal after intraportal xenotransplantation of wild-type porcine islets in immunosuppressed nonhuman primates. *Nat Med.* <https://doi.org/10.1038/nm1369>
- Hillberg AL, Oudshoorn M, Lam JBB, Kathiramanathan K (2015) Encapsulation of porcine pancreatic islets within an immunoprotective capsule comprising methacrylated glycol chitosan and alginate. *J Biomed Mater Res Appl Biomater.* <https://doi.org/10.1002/jbm.b.33185>
- Hinderer S, Layland SL, Schenke-Layland K (2016) ECM and ECM-like materials – biomaterials for applications in regenerative medicine and cancer therapy. *Adv Drug Deliv Rev.* <https://doi.org/10.1016/j.addr.2015.11.019>
- Hoesli CA, Luu M, Piret JM (2009) A novel alginate hollow fiber bioreactor process for cellular therapy applications. *Biotechnol Prog.* <https://doi.org/10.1002/btpr.260>
- Hosgood SA, Nicholson ML (2010) The role of perfluorocarbon in organ preservation. *Transplantation.* <https://doi.org/10.1097/TP.0b013e3181da6064>
- Huang X, Zhang X, Wang X, Wang C, Tang B (2012) Microenvironment of alginate-based microcapsules for cell culture and tissue engineering. *J Biosci Bioeng.* <https://doi.org/10.1016/j.jbiosc.2012.02.024>
- Huang YB, Mei J, Yu Y, Ding Y, Xia W, Yue T, Chen W, Zhou MT, Yang YJ (2018a) Comparative Decellularization and Recellularization of Normal versus Streptozotocin-induced diabetes mellitus rat pancreas. *Artif Organs.* <https://doi.org/10.1111/aor.13353>
- Huang YB, Mei J, Yu Y, Ding Y, Xia W, Yue T, Chen W, Zhou MT, Yang YJ (2018b) Comparative decellularization and recellularization of normal versus streptozotocin-induced diabetes mellitus rat pancreas. *Artif Organs.* <https://doi.org/10.1111/aor.13353>
- Hwa AJ, Weir GC (2018) Transplantation of macroencapsulated insulin-producing cells. *Curr Diab Rep.* <https://doi.org/10.1007/s11892-018-1028-y>
- Im B, Jeong J, Haque MR, Yun D, Ahn C, Eun J, Byun Y (2013) Biomaterials the effects of 8-arm-PEG-catechol/heparin shielding system and immunosuppressive drug, FK506 on the survival of intraportally allotransplanted islets. *Biomaterials.* <https://doi.org/10.1016/j.biomaterials.2012.11.028>
- Irving-Rodgers HF, Choong FJ, Hummitzsch K, Parish CR, Rodgers RJ, Simeonovic CJ (2014) Pancreatic Islet basement membrane loss and remodeling after mouse islet isolation and transplantation: impact for allograft rejection. *Cell Transplant.* <https://doi.org/10.3727/096368912X659880>
- Itkin-Ansari P, Geron I, Hao E, Demeterco C, Tyrberg B, Levine F (2003) Cell-based therapies for diabetes: progress towards a transplantable human  $\beta$  cell line. *In: Ann N Y Acad Sci* 1005(1): 138–147
- Itoh T, Iwahashi S, Shimoda M, Chujo D, Takita M, Sorelle JA, Naziruddin B, Levy MF, Matsumoto S (2011) High-mobility group box 1 expressions in hypoxia-induced damaged mouse islets. *Transplant Proc.* <https://doi.org/10.1016/j.transproceed.2011.09.100>
- Iwata H, Arima Y, Tsutsui Y (2018) Design of bioartificial pancreases from the standpoint of oxygen supply. *Artif Organs.* <https://doi.org/10.1111/aor.13106>
- Jabs N, Franklin L, Brenner MB, Gromada J, Ferrara N, Wollheim CB, Lammert E (2008) Reduced insulin secretion and content in VEGF-A deficient mouse pancreatic islets. *Exp Clin Endocrinol Diabetes.* <https://doi.org/10.1055/s-2008-1081486>
- Jacobs-Tulleneers-Thevissen D, Chintinne M, Ling Z, Gillard P, Schoonjans L, Delvaux G, Strand BL, Gorus F, Keymeulen B, Pipeleers D (2013) Sustained function of alginate-encapsulated human islet cell implants in the peritoneal cavity of mice leading to a pilot study in a type 1 diabetic patient. *Diabetologia.* <https://doi.org/10.1007/s00125-013-2906-0>
- Jansson L, Barbu A, Bodin B, Drott CJ, Espes D, Gao X, Grapensparr L, Källskog Ö, Lau J, Liljebäck H, Palm F, Quach M, Sandberg M, Strömberg V, Ullsten S, Carlsson PO (2016) Pancreatic islet blood flow and its measurement. *Ups J Med Sci.* <https://doi.org/10.3109/03009734.2016.1164769>
- Jeong JH, Yook S, Hwang JW, Jung MJ, Moon HT, Lee DY, Byun Y (2013) Synergistic effect of surface modification with poly(ethylene glycol) and immunosuppressants on repetitive pancreatic islet transplantation into antecedently sensitized rat. *Transplant Proc.* <https://doi.org/10.1016/j.transproceed.2012.02.028>

- Jiang K, Weaver JD, Li Y, Chen X, Liang J, Stabler CL (2017) Local release of dexamethasone from macroporous scaffolds accelerates islet transplant engraftment by promotion of anti-inflammatory M2 macrophages. *Biomaterials*. <https://doi.org/10.1016/j.biomaterials.2016.11.004>
- Jo J, Choi MY, Koh DS (2009) Beneficial effects of intercellular interactions between pancreatic islet cells in blood glucose regulation. *J Theor Biol*. <https://doi.org/10.1016/j.jtbi.2008.12.005>
- Jo EH, Hwang YH, Lee DY (2015) Encapsulation of pancreatic islet with HMGB1 fragment for attenuating inflammation. *Biomater Res*. <https://doi.org/10.1186/s40824-015-0042-2>
- Joglekar MV, Hardikar AA (2012) Isolation, expansion, and characterization of human islet-derived progenitor cells. *Methods Mol Biol*. [https://doi.org/10.1007/978-1-61779-815-3\\_21](https://doi.org/10.1007/978-1-61779-815-3_21)
- Joglekar MV, Joglekar VM, Hardikar AA (2009) Expression of islet-specific microRNAs during human pancreatic development. *Gene Expr Patterns*. <https://doi.org/10.1016/j.gep.2008.10.001>
- Jones JR, Zhang SC (2016) Engineering human cells and tissues through pluripotent stem cells. *Curr Opin Biotechnol*. <https://doi.org/10.1016/j.copbio.2016.03.010>
- Kang AR, Park JS, Ju J, Jeong GS, Lee SH (2014) Cell encapsulation via microtechnologies. *Biomaterials*. <https://doi.org/10.1016/j.biomaterials.2013.12.073>
- Kanno T, Göpel SO, Rorsman P, Wakui M (2002) Cellular function in multicellular system for hormone-secretion: electrophysiological aspect of studies on  $\alpha$ -,  $\beta$ - and  $\delta$ -cells of the pancreatic islet. *Neurosci Res* 42(2):79–90
- Karnieli O, Izhar-Prato Y, Bulvik S, Efrat S (2007) Generation of insulin-producing cells from human bone marrow mesenchymal stem cells by genetic manipulation. *Stem Cells*. <https://doi.org/10.1634/stemcells.2007-0164>
- Katsuki Y, Yagi H, Okitsu T, Kitago M, Tajima K, Kadota Y, Hibi T, Abe Y, Shinoda M, Itano O, Takeuchi S, Kitagawa Y (2016) Endocrine pancreas engineered using porcine islets and partial pancreatic scaffolds. *Pancreatol*. <https://doi.org/10.1016/j.pan.2016.06.007>
- Kilimnik G, Zhao B, Jo J, Periwal V, Witkowski P, Misawa R, Hara M (2011) Altered islet composition and disproportionate loss of large islets in patients with type 2 diabetes. *PLoS One*. <https://doi.org/10.1371/journal.pone.0027445>
- Kim J, Breunig MJ, Escalante LE, Bhatia N, Denu RA, Dollar BA, Stein AP, Hanson SE, Naderi N, Radek J, Haughy D, Bloom DD, Assadi-Porter FM, Hematti P (2012) Biologic and immunomodulatory properties of mesenchymal stromal cells derived from human pancreatic islets. *Cytotherapy*. <https://doi.org/10.3109/14653249.2012.684376>
- Kim Y, Kim H, Ko UH, Oh Y, Lim A, Sohn JW, Shin JH, Kim H, Han YM (2016a) Islet-like organoids derived from human pluripotent stem cells efficiently function in the glucose responsiveness in vitro and in vivo. *Sci Rep*. <https://doi.org/10.1038/srep35145>
- Kim JH, Kim HW, Cha KJ, Han J, Jang YJ, Kim DS, Kim JH (2016b) Nanotopography promotes pancreatic differentiation of human embryonic stem cells and induced pluripotent stem cells. *ACS Nano*. <https://doi.org/10.1021/acsnano.5b06985>
- Kim EJ, Kang KH, Ju JH (2017) Crispr-cas9: a promising tool for gene editing on induced pluripotent stem cells. *Korean J Intern Med*. <https://doi.org/10.3904/kjim.2016.198>
- King CC, Brown AA, Sargin I, Bratlie KM, Beckman SP (2019) Modeling of reaction-diffusion transport into a core-shell geometry. *J Theor Biol*. <https://doi.org/10.1016/j.jtbi.2018.09.026>
- Kirk K, Hao E, Lahmy R, Itkin-Ansari P (2014) Human embryonic stem cell derived islet progenitors mature inside an encapsulation device without evidence of increased biomass or cell escape. *Stem Cell Res*. <https://doi.org/10.1016/j.scr.2014.03.003>
- Kitasato H, Kai R, Ding WG, Omatsu-Kanbe M (1996) The intrinsic rhythmicity of spike-burst generation in pancreatic beta- cells and intercellular interaction within an islet. *Jpn J Physiol*. <https://doi.org/10.2170/jjphysiol.46.363>
- Klee P, Allagnat F, Pontes H, Cederroth M, Charollais A, Caille D, Britan A, Haefliger JA, Meda P (2011) Connexins protect mouse pancreatic  $\beta$  cells against apoptosis. *J Clin Invest*. <https://doi.org/10.1172/JCI40509>
- Klein D, Misawa R, Bravo-Egana V, Vargas N, Rosero S, Piroso J, Ichii H, Umland O, Zhijie J, Tsinoremans N, Ricordi C, Inverardi L, Domínguez-Bendala J, Pastori RL (2013) MicroRNA expression in alpha and Beta cells of human pancreatic islets. *PLoS One*. <https://doi.org/10.1371/journal.pone.0055064>
- Kojima H, Fujimiya M, Matsumura K, Younan P, Imaeda H, Maeda M, Chan L (2003) NeuroD-betacellulin gene therapy induces islet neogenesis in the liver and reverses diabetes in mice. *Nat Med*. <https://doi.org/10.1038/nm867>
- Kopp JL, Dubois CL, Hao E, Thorel F, Herrera PL, Sander M (2011) Progenitor cell domains in the developing and adult pancreas. *Cell Cycle* 15;10(12):1921–1927
- Korbutt GS, Elliott JF, Ao Z, Smith DK, Warnock GL, Rajotte R (1996) Large scale isolation, growth, and function of porcine neonatal islet cells. *J Clin Invest*. <https://doi.org/10.1172/JCI118649>
- Kourtzelis I, Kotlabova K, Lim JH, Mitroulis I, Ferreira A, Chen LS, Gercken B, Steffen A, Kemter E, von Ameln AK, Waskow C, Hosur K, Chatzigeorgiou A, Ludwig B, Wolf E, Hajishengallis G, Chavakis T (2016) Developmental endothelial locus-1 modulates platelet-monocyte interactions and instant blood-mediated inflammatory reaction in islet transplantation. *Thromb Haemost*. <https://doi.org/10.1160/TH15-05-0429>

- Kroon E, Martinson LA, Kadoya K, Bang AG, Kelly OG, Eliazar S, Young H, Richardson M, Smart NG, Cunningham J, Agulnick AD, D'Amour KA, Carpenter MK, Baetge EE (2008) Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. *Nat Biotechnol.* <https://doi.org/10.1038/nbt1393>
- Kuehn C, Vermette P, Fülöp T (2014) Cross talk between the extracellular matrix and the immune system in the context of endocrine pancreatic islet transplantation. A review article. *Pathol Biol.* <https://doi.org/10.1016/j.patbio.2014.01.001>
- Lammert E, Gu G, McLaughlin M, Brown D, Brekken R, Murtaugh LC, Gerber HP, Ferrara N, Melton DA (2003) Role of VEGF-A in vascularization of pancreatic islets. *Curr Biol.* [https://doi.org/10.1016/S0960-9822\(03\)00378-6](https://doi.org/10.1016/S0960-9822(03)00378-6)
- Latreille M, Herrmanns K, Renwick N, Tuschl T, Malecki MT, McCarthy MI, Owen KR, Rüllicke T, Stoffel M (2015) miR-375 gene dosage in pancreatic  $\beta$ -cells: implications for regulation of  $\beta$ -cell mass and biomarker development. *J Mol Med.* <https://doi.org/10.1007/s00109-015-1296-9>
- Lavallard V, Armanet M, Parnaud G, Meyer J, Barbieux C, Montanari E, Meier R, Morel P, Berney T, Bosco D (2016) Cell rearrangement in transplanted human islets. *FASEB J.* <https://doi.org/10.1096/fj.15-273805>
- Lee DY, Yang K, Lee S, Chae SY, Kim KW, Lee MK, Han DJ, Byun Y (2002) Optimization of monomethoxy-polyethylene glycol grafting on the pancreatic islet capsules. *J Biomed Mater Res.* <https://doi.org/10.1002/jbm.10246>
- Lee DY, Nam JH, Byun Y (2004) Effect of polyethylene glycol grafted onto islet capsules on prevention of splenocyte and cytokine attacks. *J Biomater Sci Polym Ed.* <https://doi.org/10.1163/156856204774196144>
- Lee DY, Park SJ, Nam JH, Byun Y (2006a) A combination therapy of PEGylation and immunosuppressive agent for successful islet transplantation. *J Control Release.* <https://doi.org/10.1016/j.jconrel.2005.10.023>
- Lee DY, Lee S, Nam JH, Byun Y (2006b) Minimization of immunosuppressive therapy after islet transplantation: combined action of heme oxygenase-1 and PEGylation to islet. *Am J Transplant.* <https://doi.org/10.1111/j.1600-6143.2006.01414.x>
- Lee DY, Park SJ, Nam JH, Byun Y (2006c) A new strategy toward improving immunoprotection in cell therapy for diabetes mellitus: long-functioning PEGylated islets in vivo. *Tissue Eng.* <https://doi.org/10.1089/ten.2006.12.ft-55>
- Lee SH, Hao E, Savinov AY, Geron I, Strongin AY, Itkin-Ansari P (2009) Human  $\beta$ -cell precursors mature into functional insulin-producing cells in an immunoisolation device: implications for diabetes cell therapies. *Transplantation.* <https://doi.org/10.1097/TP.0b013e31819c86ea>
- Lee KH, No DY, Kim SH, Ryoo JH, Wong SF, Lee SH (2011) Diffusion-mediated in situ alginate encapsulation of cell spheroids using microscale concave well and nanoporous membrane. *Lab Chip.* <https://doi.org/10.1039/c0lc00540a>
- Lee EM, Jung JI, Alam Z, Yi HG, Kim H, Choi JW, Hurh S, Kim YJ, Jeong JC, Yang J, Oh KH, Kim HC, Lee BC, Choi I, Cho DW, Ahn C (2018) Effect of an oxygen-generating scaffold on the viability and insulin secretion function of porcine neonatal pancreatic cell clusters. *Xenotransplantation.* <https://doi.org/10.1111/xen.12378>
- Li N, Sun G, Wang S, Wang Y, Xiu Z, Sun D, Guo X, Zhang Y, Ma X (2017) Engineering islet for improved performance by optimized reaggregation in alginate gel beads. *Biotechnol Appl Biochem.* <https://doi.org/10.1002/bab.1489>
- Liao SW, Rawson J, Omori K, Ishiyama K, Mozhdzhi D, Oancea AR, Ito T, Guan Z, Mullen Y (2013) Maintaining functional islets through encapsulation in an injectable saccharide-peptide hydrogel. *Biomaterials.* <https://doi.org/10.1016/j.biomaterials.2013.02.007>
- Liu X, Li X, Zhang N, Wen X (2015) Engineering  $\beta$ -cell islets or islet-like structures for type 1 diabetes treatment. *Med Hypotheses.* <https://doi.org/10.1016/j.mehy.2015.04.005>
- Liu Z, Hu W, He T, Dai Y, Hara H, Bottino R, Cooper DKC, Cai Z, Mou L (2017) Pig-to-primate islet xenotransplantation: past, present, and future. *Cell Transplant.* <https://doi.org/10.3727/096368917X694859>
- Liu Z, Tang M, Zhao J, Chai R, Kang J (2018) Looking into the future: toward advanced 3D biomaterials for stem-cell-based regenerative medicine. *Adv Mater.* <https://doi.org/10.1002/adma.201705388>
- Llacua A, De Haan BJ, Smink SA, De Vos P (2016) Extracellular matrix components supporting human islet function in alginate-based immunoprotective microcapsules for treatment of diabetes. *J Biomed Mater Res A.* <https://doi.org/10.1002/jbm.a.35706>
- Llacua LA, de Haan BJ, de Vos P (2018) Laminin and collagen IV inclusion in immunoisolating microcapsules reduces cytokine-mediated cell death in human pancreatic islets. *J Tissue Eng Regen Med.* <https://doi.org/10.1002/term.2472>
- Lock LT, Laychock SG, Tzanakakis ES (2011) Pseudoislets in stirred-suspension culture exhibit enhanced cell survival, propagation and insulin secretion. *J Biotechnol.* <https://doi.org/10.1016/j.jbiotec.2010.12.015>
- Loomans CJM, Williams Giuliani N, Balak J, Ringnalda F, van Gorp L, Huch M, Boj SF, Sato T, Kester L, de Sousa Lopes SMC, Roost MS, Bonner-Weir S, Engelse MA, Rabelink TJ, Heimberg H, Vries RGJ, van Oudenaarden A, Carlotti F, Clevers H, de Koning EJP (2018) Expansion of adult human pancreatic tissue yields Organoids harboring progenitor cells with endocrine differentiation potential. *Stem Cell Rep.* <https://doi.org/10.1016/j.stemcr.2018.02.005>

- Lou S, Zhang X, Zhang J, Deng J, Kong D, Li C (2017) Pancreatic islet surface bioengineering with a heparin-incorporated starPEG nanofilm. *Mater Sci Eng C*. <https://doi.org/10.1016/j.msec.2017.03.295>
- Ludwig B, Ziegler CG, Schally AV, Richter C, Steffen A, Jabs N, Funk RH, Brendel MD, Block NL, Ehrhart-Bornstein M, Bornstein SR (2010) Agonist of growth hormone-releasing hormone as a potential effector for survival and proliferation of pancreatic islets. *Proc Natl Acad Sci*. <https://doi.org/10.1073/pnas.1005098107>
- Ludwig B, Rotem A, Schmid J, Weir GC, Colton CK, Brendel MD, Neufeld T, Block NL, Yavriyants K, Steffen A, Ludwig S, Chavakis T, Reichel A, Azarov D, Zimmermann B, Maimon S, Balyura M, Rozenshtein T, Shabtay N, Vardi P, Bloch K, de Vos P, Schally AV, Bornstein SR, Barkai U (2012) Improvement of islet function in a bioartificial pancreas by enhanced oxygen supply and growth hormone releasing hormone agonist. *Proc Natl Acad Sci*. <https://doi.org/10.1073/pnas.1201868109>
- Ludwig B, Reichel A, Steffen A, Zimerman B, Schally AV, Block NL, Colton CK, Ludwig S, Kersting S, Bonifacio E, Solimena M, Gendler Z, Rotem A, Barkai U, Bornstein SR (2013) Transplantation of human islets without immunosuppression. *Proc Natl Acad Sci*. <https://doi.org/10.1073/pnas.1317561110>
- Lutolf MP, Hubbell JA (2005) Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. *Nat Biotechnol* 23 (1):47–55
- Ma S, Viola R, Sui L, Cherubini V, Barbetti F, Egli D (2018)  $\beta$  cell replacement after gene editing of a neonatal diabetes-causing mutation at the insulin locus. *Stem Cell Rep*. <https://doi.org/10.1016/j.stemcr.2018.11.006>
- Madden LR, Mortisen DJ, Sussman EM, Dupras SK, Fugate JA, Cuy JL, Hauch KD, Laflamme MA, Murry CE, Ratner BD (2010) Proangiogenic scaffolds as functional templates for cardiac tissue engineering. *Proc Natl Acad Sci*. <https://doi.org/10.1073/pnas.1006442107>
- Mahboudi H, Kazemi B, Soleimani M, Hanaee-Ahvaz H, Ghanbarian H, Bandehpour M, Enderami SE, Kehtari M, Barati G (2018) Enhanced chondrogenesis of human bone marrow mesenchymal stem cell (BMSC) on nanofiber-based polyethersulfone (PES) scaffold. *Gene*. <https://doi.org/10.1016/j.gene.2017.11.073>
- Mahou R, Zhang DKY, Vlahos AE, Sefton MV (2017) Injectable and inherently vascularizing semi-interpenetrating polymer network for delivering cells to the subcutaneous space. *Biomaterials*. <https://doi.org/10.1016/j.biomaterials.2017.03.032>
- Mao GH, Chen GA, Bai HY, Song TR, Wang YX (2009) The reversal of hyperglycaemia in diabetic mice using PLGA scaffolds seeded with islet-like cells derived from human embryonic stem cells. *Biomaterials*. <https://doi.org/10.1016/j.biomaterials.2008.12.030>
- Mao D, Zhu M, Zhang X, Ma R, Yang X, Ke T, Wang L, Li Z, Kong D, Li C (2017) A macroporous heparin-releasing silk fibroin scaffold improves islet transplantation outcome by promoting islet revascularisation and survival. *Acta Biomater*. <https://doi.org/10.1016/j.actbio.2017.06.039>
- Marchioli G, Di LA, de Koning E, Engelse M, Van Blitterswijk CA, Karperien M, Van Apeldoorn AA, Moroni L (2016) Hybrid Polycaprolactone/alginate scaffolds functionalized with VEGF to promote de novo vessel formation for the transplantation of islets of Langerhans. *Adv Healthc Mater*. <https://doi.org/10.1002/adhm.201600058>
- Mason MN, Arnold CA, Mahoney MJ (2009) Entrapped collagen type 1 promotes differentiation of embryonic pancreatic precursor cells into glucose-responsive  $\beta$  cells. *Cells Tissue Eng Part A*. <https://doi.org/10.1089/ten.TEA.2009.0148>
- Matsumoto S (2010) Islet cell transplantation for type 1 diabetes. *J Diabetes*. <https://doi.org/10.1111/j.1753-0407.2009.00048.x>
- Matsumoto S, Tan P, Baker J, Durbin K, Tomiya M, Azuma K, Doi M, Elliott RB (2014) Clinical porcine islet xenotransplantation under comprehensive regulation. *Transplant Proc*. <https://doi.org/10.1016/j.transproceed.2014.06.008>
- Matsumoto S, Tomiya M, Sawamoto O (2016) Current status and future of clinical islet xenotransplantation. 8:483–493. doi: <https://doi.org/10.1111/1753-0407.12395>
- McQuilling JP, Sittadjody S, Pendergraft S, Farney AC, Opara EC (2017) Applications of particulate oxygen-generating substances (POGS) in the bioartificial pancreas. *Biomater Sci*. <https://doi.org/10.1039/c7bm00790f>
- McReynolds J, Wen Y, Li X, Guan J, Jin S (2017) Modeling spatial distribution of oxygen in 3d culture of islet beta-cells. *Biotechnol Prog*. <https://doi.org/10.1002/btpr.2395>
- Mironov AV, Grigoryev AM, Krotova LI, Skaletsky NN, Popov VK, Sevastianov VI (2017) 3D printing of PLGA scaffolds for tissue engineering. *J Biomed Mater Res A*. <https://doi.org/10.1002/jbm.a.35871>
- Mobarra N, Soleimani M, Pakzad R, Enderami SE, Pasalar P (2018) Three-dimensional nanofibrous PLLA/PCL scaffold improved biochemical and molecular markers hiPS cell-derived insulin-producing islet-like cells. *Artif Cells Nanomed Biotechnol*. <https://doi.org/10.1080/21691401.2018.1505747>
- Moberg L, Johansson H, Lukinius A, Berne C, Foss A, Källén R, Østraat SK, Tibell A, Tufveson G, Elgue G, Nilsson Ekdahl K, Korsgren O, Nilsson B (2002) Production of tissue factor by pancreatic islet cells as a trigger of detrimental thrombotic reactions in clinical islet transplantation. *Lancet*. [https://doi.org/10.1016/S0140-6736\(02\)12020-4](https://doi.org/10.1016/S0140-6736(02)12020-4)
- Montanucci P, Pennoni I, Pescara T, Basta G, Calafiore R (2013) Treatment of diabetes mellitus with microencapsulated fetal human liver (FH-B-TPN) engineered cells. *Biomaterials*. <https://doi.org/10.1016/j.biomaterials.2013.02.026>

- Montazeri L, Hojjati-Emami S, Bonakdar S, Tahamtani Y, Hajizadeh-Saffar E, Noori-Keshtkar M, Najjar-Asl M, Ashtiani MK, Baharvand H (2016) Improvement of islet engrafts by enhanced angiogenesis and microparticle-mediated oxygenation. *Biomaterials*. <https://doi.org/10.1016/j.biomaterials.2016.02.043>
- Mooranian A, Negrulj R, Arfuso F, Al-Salami H (2016) Multicompartmental, multilayered probucol microcapsules for diabetes mellitus: formulation characterization and effects on production of insulin and inflammation in a pancreatic  $\beta$ -cell line. *Artif Cells Nanomed Biotechnol*. <https://doi.org/10.3109/21691401.2015.1069299>
- Mooranian A, Zamani N, Mikov M, Goločorbin-Kon S, Stojanovic G, Arfuso F, Al-Salami H (2018) Novel nano-encapsulation of probucol in microgels: scanning electron micrograph characterizations, buoyancy profiling, and antioxidant assay analyses. *Artif Cells Nanomed Biotechnol*:1–7. <https://doi.org/10.1080/21691401.2018.1511571>
- Morini S, Braun M, Onori P, Cicalese L, Elias G, Gaudio E, Rastellini C (2006) Morphological changes of isolated rat pancreatic islets: a structural, ultrastructural and morphometric study. *J Anat*. <https://doi.org/10.1111/j.1469-7580.2006.00620.x>
- Morozov VA, Wynyard S, Matsumoto S, Abalovich A, Denner J, Elliott R (2017) No PERV transmission during a clinical trial of pig islet cell transplantation. *Virus Res*. <https://doi.org/10.1016/j.virusres.2016.08.012>
- Motte E, Szepessy E, Suenens K, Stange G, Bomans M, Jacobs-Tulleneers-Thevisen D, Ling Z, Kroon E, Pipeleers D (2014) Composition and function of macroencapsulated human embryonic stem cell-derived implants: comparison with clinical human islet cell grafts. *Am J Physiol Endocrinol Metab*. <https://doi.org/10.1152/ajpendo.00219.2014>
- Murray HE, Paget MB, Downing R (2005) Preservation of glucose responsiveness in human islets maintained in a rotational cell culture system. *Mol Cell Endocrinol*. <https://doi.org/10.1016/j.mce.2005.03.014>
- Nabavimanes MM, Hashemi-Najafabadi S, Vasheghani-Farahani E (2015) Islets immunoisolation using encapsulation and PEGylation, simultaneously, as a novel design. *J Biosci Bioeng*. <https://doi.org/10.1016/j.jbiosc.2014.09.023>
- Nadri S, Barati G, Mostafavi H, Esmaeilzadeh A, Enderami SE (2017) Differentiation of conjunctiva mesenchymal stem cells into secreting islet beta cells on plasma treated electrospun nanofibrous scaffold. *Artif Cells Nanomed Biotechnol*. <https://doi.org/10.1080/21691401.2017.1416391>
- Nassiri Mansour R, Barati G, Soleimani M, Ghoraeian P, Nouri Aleagha M, Kehtari M, Mahboudi H, Hosseini F, Hassannia H, Abazari MF, Enderami SE (2018) Generation of high-yield insulin producing cells from human-induced pluripotent stem cells on polyethersulfone nanofibrous scaffold. *Artif Cells Nanomed Biotechnol*. <https://doi.org/10.1080/21691401.2018.1434663>
- Navarro-Tableros V, Herrera Sanchez MB, Figliolini F, Romagnoli R, Tetta C, Camussi G (2015) Recellularization of rat liver scaffolds by human liver stem cells. *Tissue Eng Part A*. <https://doi.org/10.1089/ten.tea.2014.0573>
- Navarro-tableros V, Gai C, Gomez Y, Giunti S, Pasquino C, Deregibus MC, Tapparo M, Pitino A, Tetta C, Brizzi MF, Ricordi C, Camussi G (2018) Islet-like structures generated in vitro from adult human liver stem cells revert hyperglycemia in diabetic SCID mice. *Stem Cell Rev Rep*. <https://doi.org/10.1007/s12015-018-9845-6>
- Nelson TJ, Behfar A, Yamada S, Martinez-Fernandez A, Terzic A (2009) Stem cell platforms for regenerative medicine. *Clin Transl Sci*. <https://doi.org/10.1111/j.1752-8062.2009.00096.x>
- Neufeld T, Ludwig B, Barkai U, Weir GC, Colton CK, Evron Y, Balyura M, Yavriyants K, Zimmermann B, Azarov D, Maimon S, Shabtay N, Rozenshtein T, Lorber D, Steffen A, Willenz U, Bloch K, Vardi P, Taube R, de Vos P, Lewis EC, Bornstein SR, Rotem A (2013) The efficacy of an immunoisolating membrane system for islet xenotransplantation in minipigs. *PLoS One*. <https://doi.org/10.1371/journal.pone.0070150>
- Nikolova G, Jabs N, Konstantinova I, Domogatskaya A, Tryggvason K, Sorokin L, Fässler R, Gu G, Gerber HP, Ferrara N, Melton DA, Lammert E (2006) The vascular basement membrane: a niche for insulin gene expression and  $\beta$  cell proliferation. *Dev Cell*. <https://doi.org/10.1016/j.devcel.2006.01.015>
- Nikravesh N, Cox SC, Ellis MJ, Grover LM (2017) Encapsulation and fluidization maintains the viability and glucose sensitivity of Beta-cells. *ACS Biomater Sci Eng*. <https://doi.org/10.1021/acsbomaterials.7b00191>
- Nilsson B, Ekdahl KN, Korsgren O (2011) Control of instant blood-mediated inflammatory reaction to improve islets of Langerhans engraftment. *Curr Opin Organ Transplant*. <https://doi.org/10.1097/MOT.0b013e32834c2393>
- Nir T, Melton DA, Dor Y (2007) Recovery from diabetes in mice by  $\beta$  cell regeneration. *J Clin Invest*. <https://doi.org/10.1172/JCI32959>
- Olsson R, Olerud J, Petterson U, Carlsson PO (2011) Increased numbers of low-oxygenated pancreatic islets after intraportal islet transplantation. *Diabetes* 60:2350–2353. <https://doi.org/10.2337/db09-0490>
- Onoe H, Okitsu T, Itou A, Kato-Negishi M, Gojo R, Kiriya D, Sato K, Miura S, Iwanaga S, Kuribayashi-Shigetomi K, Matsunaga YT, Shimoyama Y, Takeuchi S (2013) Metre-long cell-laden microfibrils exhibit tissue morphologies and functions. *Nat Mater*. <https://doi.org/10.1038/nmat3606>
- Orci L, Malaisse-Lagae F, Ravazzola M, Rouiller D, Renold AE, Perrelet A, Unger R (1975) A morphological basis for intercellular communication between  $\alpha$  and  $\beta$  cells in the endocrine pancreas. *J Clin Invest*. <https://doi.org/10.1172/JCI108154>

- Orive G, Santos E, Poncelet D, Hernández RM, Pedraz JL, Wahlberg LU, De Vos P, Emerich D (2015) Cell encapsulation: technical and clinical advances. *Trends Pharmacol Sci* 36:537–546. <https://doi.org/10.1016/j.tips.2015.05.003>
- Ouziel-Yahalom L, Zalzman M, Anker-Kitai L, Knoller S, Bar Y, Glandt M, Herold K, Efrat S (2006) Expansion and redifferentiation of adult human pancreatic islet cells. *Biochem Biophys Res Commun*. <https://doi.org/10.1016/j.bbrc.2005.12.187>
- Pagliuca FW, Melton DA (2013) How to make a functional  $\beta$ -cell. *Development* 140:2472–2483. <https://doi.org/10.1242/dev.093187>
- Pagliuca FW, Millman JR, Gürtler M, Segel M, Van Dervort A, Ryu JH, Peterson QP, Greiner D, Melton DA (2014) Generation of functional human pancreatic  $\beta$  cells in vitro. *Cell*. <https://doi.org/10.1016/j.cell.2014.09.040>
- Paraskevas S, Maysinger D, Wang R, Duguid WP, Rosenberg L (2000) Cell loss in isolated human islets occurs by apoptosis. *Pancreas*. <https://doi.org/10.1097/00006676-200004000-00008>
- Park CH, Woo KM (2018) Fibrin-based biomaterial applications in tissue engineering and regenerative medicine. *Adv Exp Med Biol*. [https://doi.org/10.1007/978-981-13-0445-3\\_16](https://doi.org/10.1007/978-981-13-0445-3_16).doi:10.1007/978-981-13-0445-3\_16
- Park HS, Kim JW, Lee SH, Yang HK, Ham DS, Sun CL, Hong TH, Khang G, Park CG, Yoon KH (2017) Antifibrotic effect of rapamycin containing polyethylene glycol-coated alginate microcapsule in islet xenotransplantation. *J Tissue Eng Regen Med*. <https://doi.org/10.1002/term.2029>
- Päth G, Perakakis N, Mantzoros CS, Seufert J (2019) Stem cells in the treatment of diabetes mellitus-focus on mesenchymal stem cells. *Metabolism*. <https://doi.org/10.1016/j.metabol.2018.10.005>
- Pedraza E, Coronel MM, Fraker CA, Ricordi C, Stabler CL (2012) Preventing hypoxia-induced cell death in beta cells and islets via hydrolytically activated, oxygen-generating biomaterials. *Proc Natl Acad Sci*. <https://doi.org/10.1073/pnas.1113560109>
- Pepper AR, Gala-Lopez B, Pawlick R, Merani S, Kin T, Shapiro AMJ (2015) A prevascularized subcutaneous device-less site for islet and cellular transplantation. *Nat Biotechnol*. <https://doi.org/10.1038/nbt.3211>
- Pepper AR, Bruni A, Shapiro AMJ (2018) Clinical islet transplantation: is the future finally now? *Curr Opin Organ Transplant*. <https://doi.org/10.1097/MOT.0000000000000546>
- Perignon C, Ongmayeb G, Neufeld R, Frere Y, Poncelet D (2015) Microencapsulation by interfacial polymerisation: membrane formation and structure. *J Microencapsul*. <https://doi.org/10.3109/02652048.2014.950711>
- Petry F, Weidner T, Czermak P, Salzig D (2018) Three-dimensional bioreactor technologies for the cocultivation of human mesenchymal stem/stromal cells and beta cells. *Stem Cells Int*. <https://doi.org/10.1155/2018/2547098>
- Piemonti L, Everly MJ, Maffi P, Scavini M, Poli F, Nano R, Cardillo M, Melzi R, Mercalli A, Sordi V, Lampasona V, De Arias AE, Scalapogna M, Bosi E, Bonifacio E, Secchi A, Terasaki PI (2013) Alloantibody and autoantibody monitoring predicts islet transplantation outcome in human type 1 diabetes. *Diabetes*. <https://doi.org/10.2337/db12-1258>
- Pipeleers D, Chintinne M, Denys B, Martens G, Keymeulen B, Gorus F (2008) Restoring a functional  $\beta$ -cell mass in diabetes. *Diabetes Obes Metab*. <https://doi.org/10.1111/j.1463-1326.2008.00941.x>
- Rana D, Zreiqat H, Benkirane-Jessel N, Ramakrishna S, Ramalingam M (2017) Development of decellularized scaffolds for stem cell-driven tissue engineering. *J Tissue Eng Regen Med*. <https://doi.org/10.1002/term.2061>
- Ratcliffe A, Niklason LE (2002) Bioreactors and bioprocessing for tissue engineering. *Ann N Y Acad Sci* 961(1):210–215
- Ravassard P, Hazhou Y, Pechberty S, Bricout-Neveu E, Armanet M, Czernichow P, Scharfmann R (2011) A genetically engineered human pancreatic  $\beta$  cell line exhibiting glucose-inducible insulin secretion. *J Clin Invest*. <https://doi.org/10.1172/JCI58447>
- Rengifo HR, Giraldo JA, Labrada I, Stabler CL (2014) Long-term survival of allograft murine islets coated via covalently stabilized polymers. *Adv Healthc Mater*. <https://doi.org/10.1002/adhm.201300573>
- Richards OC, Raines SM, Attie AD (2010) The role of blood vessels, endothelial cells, and vascular pericytes in insulin secretion and peripheral insulin action. *Endocr Rev*. <https://doi.org/10.1210/er.2009-0035>
- Ricordi C, Fraker C, Szust J, Al-Abdullah I, Poggioli R, Kirlow T, Khan A, Alejandro R (2003) Improved human islet isolation outcome from marginal donors following addition of oxygenated perfluorocarbon to the cold-storage solution. *Transplantation*. <https://doi.org/10.1097/01.TP.0000058813.95063.7A>
- Ricordi C, Goldstein JS, Balamurugan AN, Szot GL, Kin T, Liu C, Czarniecki CW, Barbaro B, Bridges ND, Cano J, Clarke WR, Eggerman TL, Hunsicker LG, Kaufman DB, Khan A, Lafontant DE, Linetsky E, Luo X, Markmann JF, Naji A, Korsgren O, Oberholzer J, Turgeon NA, Brandhorst D, Friberg AS, Lei J, Wang LJ, Wilhelm JJ, Willits J, Zhang X, Hering BJ, Posselt AM, Stock PG, Shapiro AMJ (2016) National institutes of health-sponsored clinical islet transplantation consortium phase 3 trial: manufacture of a complex cellular product at eight processing facilities. *Diabetes*. <https://doi.org/10.2337/db16-0234>
- Rios PD, Zhang X, Luo X, Shea LD (2016) Mold-casted non-degradable, islet macro-encapsulating hydrogel devices for restoration of normoglycemia in diabetic mice. *Biotechnol Bioeng*. <https://doi.org/10.1002/bit.26005>

- Rios PD, Skoumal M, Liu J, Youngblood R, Kniazeva E, Garcia AJ, Shea LD (2018) Evaluation of encapsulating and microporous nondegradable hydrogel scaffold designs on islet engraftment in rodent models of diabetes. *Biotechnol Bioeng.* <https://doi.org/10.1002/bit.26741>
- Rogers SA, Tripathi P, Mohanakumar T, Liapis H, Chen F, Talcott MR, Faulkner C, Hammerman MR (2011) Engraftment of cells from porcine islets of langerhans following transplantation of pig pancreatic primordia in non-immunosuppressed diabetic rhesus macaques. *Organogenesis.* <https://doi.org/10.4161/org.7.3.16522>
- Rorsman P, Ashcroft FM (2018) Pancreatic  $\beta$ -cell electrical activity and insulin secretion: of mice and men. *Physiol Rev.* <https://doi.org/10.1152/physrev.00008.2017>
- Russ HA, Bar Y, Ravassard P, Efrat S (2008) In vitro proliferation of cells derived from adult human  $\beta$ -cells revealed by cell-lineage tracing. *Diabetes.* <https://doi.org/10.2337/db07-1283>
- Russ HA, Ravassard P, Kerr-Conte J, Pattou F, Efrat S (2009) Epithelial-mesenchymal transition in cells expanded in vitro from lineage-traced adult human pancreatic beta cells. *PLoS One.* <https://doi.org/10.1371/journal.pone.0006417>
- Russ HA, Sintov E, Anker-Kitai L, Friedman O, Lenz A, Toren G, Farhy C, Pasmanik-Chor M, Oron-Karni V, Ravassard P, Efrat S (2011) Insulin-producing cells generated from dedifferentiated human pancreatic beta cells expanded in vitro. *PLoS One.* <https://doi.org/10.1371/journal.pone.0025566>
- Rutter GA, Hodson DJ (2015) Beta cell connectivity in pancreatic islets: a type 2 diabetes target? *Cell Mol Life Sci.* <https://doi.org/10.1007/s00018-014-1755-4>
- Rutzky LP, Bilinski S, Kloc M, Phan T, Zhang H, Katz SM, Stepkowski SM (2002) Microgravity culture condition reduces immunogenicity and improves function of pancreatic islets. *Transplantation.* <https://doi.org/10.1097/00007890-200207150-00004>
- Ryan AJ, O'Neill HS, Duffy GP, O'Brien FJ (2017) Advances in polymeric islet cell encapsulation technologies to limit the foreign body response and provide immunoisolation. *Curr Opin Pharmacol.* <https://doi.org/10.1016/j.coph.2017.07.013>
- Saeki Y, Ishiyama K, Ishida N, Tanaka Y, Ohdan H (2017) Role of natural killer cells in the innate immune system after intraportal islet transplantation in mice. *Transplant Proc.* <https://doi.org/10.1016/j.transproceed.2016.10.010>
- Safley SA, Kenyon NS, Berman DM, Barber GF, Willman M, Duncanson S, Iwakoshi N, Holdcraft R, Gazda L, Thompson P, Badell IR, Sambanis A, Ricordi C, Weber CJ (2018a) Microencapsulated adult porcine islets transplanted intraperitoneally in streptozotocin-diabetic non-human primates. *Xenotransplantation.* <https://doi.org/10.1111/xen.12095>
- Safley SA, Kenyon NS, Berman DM, Barber GF, Willman M, Duncanson S, Iwakoshi N, Holdcraft R, Gazda L, Thompson P, Badell IR, Sambanis A, Ricordi C, Weber CJ (2018b) Microencapsulated adult porcine islets transplanted intraperitoneally in streptozotocin-diabetic non-human primates. *Xenotransplantation.* <https://doi.org/10.1111/xen.12450>
- Salvay DM, Rives CB, Zhang X, Chen F, Kaufman DB, Lowe WL, Shea LD (2008) Extracellular matrix protein-coated scaffolds promote the reversal of diabetes after extrahepatic islet transplantation. *Transplantation.* <https://doi.org/10.1097/TP.0b013e31816fc0ea>
- Sama AE, D'Amore J, Ward MF, Chen G, Wang H (2004) Bench to bedside: HMGB1 – a novel proinflammatory cytokine and potential therapeutic target for septic patients in the emergency department. *Acad Emerg Med*
- Samy KP, Martin BM, Turgeon NA, Kirk AD (2014) Islet cell xenotransplantation: a serious look toward the clinic. *Xenotransplantation.* <https://doi.org/10.1111/xen.12095>
- Samy KP, Davis RP, Gao Q, Martin BM, Song M, Cano J, Farris AB, McDonald A, Gall EK, Dove CR, Leopardi FV, How T, Williams KD, Devi GR, Collins BH, Kirk AD (2018) Early barriers to neonatal porcine islet engraftment in a dual transplant model. *Am J Transplant.* <https://doi.org/10.1111/ajt.14601>
- Santos-Silva JC, Carvalho CP de F, de Oliveira RB, Boschero AC, Collares-Buzato CB (2012) Cell-to-cell contact dependence and junctional protein content are correlated with in vivo maturation of pancreatic beta cells. *Can J Physiol Pharmacol.* <https://doi.org/10.1139/y2012-064>
- Scavuzzo MA, Teaw J, Yang DBM (2018) Generation of scaffold-free, three-dimensional insulin expressing pancreatoids from mouse pancreatic progenitors in vitro. *J Vis Exp.* <https://doi.org/10.3791/57599>
- Scharfmann R, Pechberty S, Hazhouz Y, Von Bülow M, Bricout-Neveu E, Grenier-Godard M, Guez F, Rachdi L, Lohmann M, Czernichow P, Ravassard P (2014) Development of a conditionally immortalized human pancreatic  $\beta$  cell line. *J Clin Invest.* <https://doi.org/10.1172/JCI72674>
- Scharp DW, Marchetti P (2014) Encapsulated islets for diabetes therapy: history, current progress, and critical issues requiring solution. *Adv Drug Deliv Rev.* <https://doi.org/10.1016/j.addr.2013.07.018>
- Schuetz C, Anazawa T, Cross SE, Labriola L, Meier RPH, Redfield RR, Scholz H, Stock PG, Zammit NW (2018)  $\beta$  cell replacement therapy: the next 10 years. *Transplantation.* <https://doi.org/10.1097/TP.0000000000001937>
- Schwarz RP, Goodwin TJ, Wolf DA (1992) Cell culture for three-dimensional modeling in rotating-wall vessels: an application of simulated microgravity. *J Tissue Cult Methods.* <https://doi.org/10.1007/BF01404744>
- Schweicher J, Nyitray C, Desai TA (2014) Membranes to achieve immunoprotection of transplanted islets. *Front Biosci* 19:49–76

- Shalaly ND, Ria M, Johansson U, Åvall K, Berggren PO, Hedhammar M (2016) Silk matrices promote formation of insulin-secreting islet-like clusters. *Biomaterials*. <https://doi.org/10.1016/j.biomaterials.2016.03.006>
- Shapiro AMJ, Ricordi C, Hering BJ, Auchincloss H, Lindblad R, Robertson RP, Secchi A, Brendel MD, Berney T, Brennan DC, Cagliero E, Alejandro R, Ryan EA, DiMercurio B, Morel P, Polonsky KS, Reems J-A, Bretzel RG, Bertuzzi F, Froud T, Kandaswamy R, Sutherland DER, Eisenbarth G, Segal M, Preiksaitis J, Korbutt GS, Barton FB, Viviano L, Seyfert-Margolis V, Bluestone J, Lakey JRT (2006) International trial of the Edmonton protocol for islet transplantation. *N Engl J Med*. <https://doi.org/10.1056/NEJMoa061267>
- Shapiro AMJ, Pokrywczynska M, Ricordi C (2017) Clinical pancreatic islet transplantation. *Nat Rev Endocrinol*. <https://doi.org/10.1038/nrendo.2016.178>
- Sharp J, Vermette P (2017) An in-situ glucose-stimulated insulin secretion assay under perfusion bioreactor conditions. *Biotechnol Prog*. <https://doi.org/10.1002/btpr.2407>
- Shen Y, Hou Y, Yao S, Huang P, Yobas L (2015) In vitro epithelial Organoid generation induced by substrate nanotopography. *Sci Rep*. <https://doi.org/10.1038/srep09293>
- Skrzypek K, Nibbelink MG, Karbaat LP, Karperien M, van Apeldoorn A, Stamatialis D (2018a) An important step towards a prevascularized islet macroencapsulation device—effect of micropatterned membranes on development of endothelial cell network. *J Mater Sci Mater Med*. <https://doi.org/10.1007/s10856-018-6102-0>
- Skrzypek K, Barrera YB, Groth T, Stamatialis D (2018b) Endothelial and beta cell composite aggregates for improved function of a bioartificial pancreas encapsulation device. *Int J Artif Organs*. <https://doi.org/10.1177/0391398817752295>
- Smink AM, Li S, Hertsig DT, De Haan BJ, Schwab L, Van Apeldoorn AA, De Koning E, Faas MM, Lakey JRT, De Vos P (2017) The efficacy of a Prevascularized, retrievable poly(D,L,-lactide-co-ε-caprolactone) subcutaneous scaffold as transplantation site for pancreatic islets. *Transplantation*. <https://doi.org/10.1097/TP.0000000000001663>
- Song S, Roy S (2016) Progress and challenges in macroencapsulation approaches for type 1 diabetes (T1D) treatment: cells, biomaterials, and devices. *Biotechnol Bioeng*. <https://doi.org/10.1002/bit.25895>
- Soon-Shiong P, Heintz RE, Merideth N, Yao QX, Yao Z, Zheng T, Murphy M, Moloney MK, Schmehl M, Harris M, Mendez R, Mendez R, Sandford PA (1994) Insulin independence in a type 1 diabetic patient after encapsulated islet transplantation. *Lancet*. [https://doi.org/10.1016/S0140-6736\(94\)90067-1](https://doi.org/10.1016/S0140-6736(94)90067-1)
- Sörenby AK, Kumagai-Braesch M, Sharma A, Hultenby KR, Wernerson AM, Tibell AB (2008) Preimplantation of an immunoprotective device can lower the curative dose of islets to that of free islet transplantation—studies in a rodent model. *Transplantation*. <https://doi.org/10.1097/TP.0b013e31817efc78>
- Steele JAM, Hallé JP, Poncelet D, Neufeld RJ (2014) Therapeutic cell encapsulation techniques and applications in diabetes. *Adv Drug Deliv Rev*. <https://doi.org/10.1016/j.addr.2013.09.015>
- Steffens D, Braghirolli DI, Maurmann N, Pranke P (2018) Update on the main use of biomaterials and techniques associated with tissue engineering. *Drug Discov Today*. <https://doi.org/10.1016/j.drudis.2018.03.013>
- Stendahl JC, Kaufman DB, Stupp SI (2009) Extracellular matrix in pancreatic islets: relevance to scaffold design and transplantation. *Cell Transplant*. <https://doi.org/10.3727/096368909788237195>
- Stephens CH, Orr KS, Acton AJ, Tersey SA, Mirmira RG, Considine RV, Voytik-Harbin SL (2018) In situ type I oligomeric collagen macroencapsulation promotes islet longevity and function in vitro and in vivo. *Am J Physiol Endocrinol Metab*. <https://doi.org/10.1152/ajpendo.00073.2018>
- Sun Y, Ma X, Zhou D, Vacek I, Sun AM (1996) Normalization of diabetes in spontaneously diabetic cynomolgus monkeys by xenografts of microencapsulated porcine islets without immunosuppression. *J Clin Invest*. <https://doi.org/10.1172/JCI118929>
- Sweet IR, Yanay O, Waldron L, Gilbert M, Fuller JM, Tupling T, Lernmark A, Osborne WRA (2008) Treatment of diabetic rats with encapsulated islets. *J Cell Mol Med* 1. <https://doi.org/10.1111/j.1582-4934.2008.00322.x>
- Swinehart IT, Badylak SF (2016) Extracellular matrix bioscaffolds in tissue remodeling and morphogenesis. *Dev Dyn*. <https://doi.org/10.1002/dvdy.24379>
- Szot GL, Yadav M, Lang J, Kroon E, Kerr J, Kadoya K, Brandon EP, Baetge EE, Bour-Jordan H, Bluestone JA (2015) Tolerance induction and reversal of diabetes in mice transplanted with human embryonic stem cell-derived pancreatic endoderm. *Cell Stem Cell*. <https://doi.org/10.1016/j.stem.2014.12.001>
- Tanaka H, Tanaka S, Sekine K, Kita S, Okamura A, Takebe T, Zheng YW, Ueno Y, Tanaka J, Taniguchi H (2013) The generation of pancreatic β-cell spheroids in a simulated microgravity culture system. *Biomaterials*. <https://doi.org/10.1016/j.biomaterials.2013.04.003>
- Theocharis AD, Skandalis SS, Gialeli C, Karamanos NK (2016) Extracellular matrix structure. *Adv Drug Deliv Rev*. <https://doi.org/10.1016/j.addr.2015.11.001>
- Thompson P, Badell IR, Lowe M, Cano J, Song M, Leopardi F, Avila J, Ruhil R, Strobert E, Korbutt G, Rayat G, Rajotte R, Iwakoshi N, Larsen CP, Kirk AD (2011) Islet xenotransplantation using gal-deficient neonatal donors improves engraftment and function. *Am J Transplant*. <https://doi.org/10.1111/j.1600-6143.2011.03720.x>
- Tibell A, Rafael E, Wennberg L, Nordenström J, Bergström M, Geller RL, Loudovaris T, Johnson RC, Brauker JH, Neuenfeldt S, Wernerson A (2001)



- Survival of macroencapsulated allogeneic parathyroid tissue one year after transplantation in nonimmunosuppressed humans. *Cell Transplant*
- Tomei AA, Villa C, Ricordi C (2015) Development of an encapsulated stem cell-based therapy for diabetes. *Expert Opin Biol Ther*. <https://doi.org/10.1517/14712598.2015.1055242>
- Trivedi N, Steil GM, Colton CK, Bonner-Weir S, Weir GC (2000) Improved vascularization of planar membrane diffusion devices following continuous infusion of vascular endothelial growth factor. *Cell Transplant*. <https://doi.org/10.1177/096368970000900114>
- Tuch BE, Keogh GW, Williams LJ, Wu W, Foster JL, Vaithilingam V, Philips R (2009) Safety and viability of microencapsulated human islets transplanted into diabetic humans. *Diabetes Care*. <https://doi.org/10.2337/dc09-0744>
- Vaithilingam V, Kollarikova G, Qi M, Lacik I, Oberholzer J, Guillemin GJ, Tuch BE (2011) Effect of prolonged gelling time on the intrinsic properties of barium alginate microcapsules and its biocompatibility. *J Microencapsul*. <https://doi.org/10.3109/02652048.2011.586067>
- Van Der Laan LJW, Lockey C, Griffeth BC, Frasier FS, Wilson CA, Onlons DE, Hering BJ, Long Z, Otto E, Torbett BE, Salomon DR (2000) Infection by porcine endogenous retrovirus after islet xenotransplantation in SCID mice. *Nature*. <https://doi.org/10.1038/35024089>
- van der Meulen T, Mawla AM, DiGruccio MR, Adams MW, Nies V, Dölleman S, Liu S, Ackermann AM, Cáceres E, Hunter AE, Kaestner KH, Donaldson CJ, Huising MO (2017) Virgin beta cells persist throughout life at a Neogenic niche within pancreatic islets. *Cell Metab*. <https://doi.org/10.1016/j.cmet.2017.03.017>
- Vegas AJ, Veiseh O, Gürtler M, Millman JR, Pagliuca FW, Bader AR, Doloff JC, Li J, Chen M, Olejnik K, Tam HH, Jhunjhunwala S, Langan E, Aresta-Dasilva S, Gandham S, McGarrigle JJ, Bochenek MA, Hollister-Lock J, Oberholzer J, Greiner DL, Weir GC, Melton DA, Langer R, Anderson DG (2016) Long-term glycemic control using polymer-encapsulated human stem cell-derived beta cells in immune-competent mice. *Nat Med*. <https://doi.org/10.1038/nm.4030>
- Veiseh O, Doloff JC, Ma M, Vegas AJ, Tam HH, Bader AR, Li J, Langan E, Wyckoff J, Loo WS, Jhunjhunwala S, Chiu A, Siebert S, Tang K, Hollister-Lock J, Aresta-Dasilva S, Bochenek M, Mendoza-Elias J, Wang Y, Qi M, Lavin DM, Chen M, Dholakia N, Thakrar R, Lacik I, Weir GC, Oberholzer J, Greiner DL, Langer R, Anderson DG (2015) Size- and shape-dependent foreign body immune response to materials implanted in rodents and non-human primates. *Nat Mater*. <https://doi.org/10.1038/nmat4290>
- Vériter S, Gianello P, Igarashi Y, Beaurin G, Ghyselinck A, Aouassar N, Jordan B, Gallez B, Dufrene D (2014) Improvement of subcutaneous bioartificial pancreas vascularization and function by coencapsulation of pig islets and mesenchymal stem cells in primates. *Cell Transplant*. <https://doi.org/10.3727/096368913X663550>
- Vishwakarma SK, Lakkireddy C, Bardia A, Raju N, Paspala SAB, Habeeb MA, Khan AA (2018) Molecular dynamics of pancreatic transcription factors in bioengineered humanized insulin producing neoorgan. *Gene*. <https://doi.org/10.1016/j.gene.2018.07.006>
- Wan J, Huang Y, Zhou P, Guo Y, Wu C, Zhu S, Wang Y, Wang L, Lu Y, Wang Z (2017) Culture of iPSCs derived pancreatic  $\beta$ -like cells in vitro using decellularized pancreatic scaffolds: a preliminary trial. *Biomed Res Int*. <https://doi.org/10.1155/2017/4276928>
- Wang Q, Li S, Xie Y, Yu W, Xiong Y, Ma X, Yuan Q (2006) Cytoskeletal reorganization and repolarization of hepatocarcinoma cells in APA microcapsule to mimic native tumor characteristics. *Hepatol Res*. <https://doi.org/10.1016/j.hepres.2006.03.003>
- Wang Y, Lanzoni G, Carpino G, Bin CC, Dominguez-Bendala J, Wauthier E, Cardinale V, Oikawa T, Pileggi A, Gerber D, Furth ME, Alvaro D, Gaudio E, Invernardi L, Reid LM (2013) Biliary tree stem cells, precursors to pancreatic committed progenitors: evidence for possible life-long pancreatic organogenesis. *Stem Cells*. <https://doi.org/10.1002/stem.1460>
- Wang Y, Zhong J, Zhang X, Liu Z, Yang Y, Gong Q, Ren B (2016) The role of HMGB1 in the pathogenesis of type 2 diabetes. *J Diabetes Res* 2016:2543268. <https://doi.org/10.1155/2016/2543268>
- Wang W, Jin S, Ye K (2017) Development of islet organoids from H9 human embryonic stem cells in biomimetic 3D scaffolds. *Stem Cells Dev*. <https://doi.org/10.1089/scd.2016.0115>
- Weaver JD, Headen DM, Hunckler MD, Coronel MM, Stabler CL, García AJ (2018) Design of a vascularized synthetic poly(ethylene glycol) macroencapsulation device for islet transplantation. *Biomaterials*. <https://doi.org/10.1016/j.biomaterials.2018.04.047>
- Wei C, Geras-Raaka E, Marcus-Samuels B, Oron Y, Gershengorn MC (2006) Trypsin and thrombin accelerate aggregation of human endocrine pancreas precursor cells. *J Cell Physiol*. <https://doi.org/10.1002/jcp.20459>
- Welsch CA, Rust WL, Csete M1 (2018) Concise review : lessons learned from islet transplant clinical trials in developing stem cell therapies for type 1 diabetes. *Stem Cells Transl Med*. <https://doi.org/10.1002/sctm.18-0156>
- Wilson JT, Cui W, Chaikof EL (2008) Layer-by-layer assembly of a conformal nanothin PEG coating for intraportal islet transplantation. *Nano Lett*. <https://doi.org/10.1021/nl808694q>
- Wojtuszczyńska A, Armanet M, Morel P, Berney T, Bosco D (2008) Insulin secretion from human beta cells is heterogeneous and dependent on cell-to-cell contacts. *Diabetologia*. <https://doi.org/10.1007/s00125-008-1103-z>

- Wu H, Avgoustiniatos ES, Swette L, Bonner-Weir S, Weir GC, Colton CK (1999) In situ electrochemical oxygen generation with an immunoisolation device. *Ann N Y Acad Sci* 875(1):105–125
- Wu XH, Liu CP, Xu KF, Mao XD, Zhu J, Jiang JJ, Cui D, Zhang M, Xu Y, Liu C (2007) Reversal of hyperglycemia in diabetic rats by portal vein transplantation of islet-like cells generated from bone marrow mesenchymal stem cells. *World J Gastroenterol*. <https://doi.org/10.3748/wjg.v13.i24.3342>
- Wynyard S, Nathu D, Garkavenko O, Denner J, Elliott R (2014) Microbiological safety of the first clinical pig islet xenotransplantation trial in New Zealand. *Xenotransplantation*. <https://doi.org/10.1111/xen.12102>
- Xie M, Ye H, Wang H, Charpin-El Hamri G, Lormeau C, Saxena P, Stelling J, Fussenegger M (2016)  $\beta$ -cell-mimetic designer cells provide closed-loop glycemic control. *Science*. <https://doi.org/10.1126/science.aaf4006>
- Yim EKF, Darling EM, Kulangara K, Guilak F, Leong KW (2010) Nanotopography-induced changes in focal adhesions, cytoskeletal organization, and mechanical properties of human mesenchymal stem cells. *Biomaterials*. <https://doi.org/10.1016/j.biomaterials.2009.10.037>
- Zarekhalili Z, Bahrami SH, Ranjbar-Mohammadi M, Milan PB (2017) Fabrication and characterization of PVA/Gum tragacanth/PCL hybrid nanofibrous scaffolds for skin substitutes. *Int J Biol Macromol*. <https://doi.org/10.1016/j.ijbiomac.2016.10.042>
- Zhang X, Wang W, Xie Y, Zhang Y, Wang X, Guo X, Ma X (2006) Proliferation, viability, and metabolism of human tumor and normal cells cultured in microcapsule. *Appl Biochem Biotechnol*. <https://doi.org/10.1385/ABAB:134:1:61>
- Zhao M, Amiel SA, Christie MR, Muesan P, Srinivasan P, Littlejohn W, Rela M, Arno M, Heaton N, Huang GC (2007) Evidence for the presence of stem cell-like progenitor cells in human adult pancreas. *J Endocrinol*. <https://doi.org/10.1677/JOE-07-0436>
- Zhou Q, Melton DA (2018) Pancreas regeneration. *Nature*. <https://doi.org/10.1038/s41586-018-0088-0>
- Zhou P, Guo Y, Huang Y, Zhu M, Fan X, Wang L, Wang Y, Zhu S, Xu T, Wu D, Lu Y, Wang Z (2016) The dynamic three-dimensional culture of islet-like clusters in decellularized liver scaffolds. *Cell Tissue Res*. <https://doi.org/10.1007/s00441-015-2356-8>
- Zimmermann H, Shirley SG, Zimmermann U (2007) Alginate-based encapsulation of cells: past, present, and future. *Curr Diab Rep*
- Zuber SM, Grikscheit TC (2018) Stem cells for babies and their surgeons: the future is now. *J Pediatr Surg*. <https://doi.org/10.1016/j.jpedsurg.2018.10.027>
- Zulewski H, Abraham EJ, Gerlach MJ, Daniel PB, Moritz W, Müller B, Vallejo M, Thomas MK, Habener JF (2001) Multipotential nestin-positive stem cells isolated from adult pancreatic islets differentiate ex vivo into pancreatic endocrine, exocrine, and hepatic phenotypes. *Diabetes*. <https://doi.org/10.2337/diabetes.50.3.521>