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Review

Pancreatic islet (of Langerhans) revisited

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Summary. One hundred and fifty years ago, Paul Langerhans described what would come to be known as pancreatic 'islet of Langerhans'. Since then, we have accumulated knowledge about the pancreatic islet, the cells that exist there and the hormones secreted by these cells. The increasing prevalence of obesity, diabetes and Alzheimer's disease in the population (three conditions that are linked to pancreatic islet function), the islet has been playing a significant role in endocrinological and metabolic studies searching how we can protect the pancreatic islet and its cell content, or how we can regenerate it. This review will be interested in the most recent and relevant aspects of knowledge regarding the pancreatic islet, always mentioning the evolution of knowledge and future perspectives for the treatment of diabetes and Alzheimer's disease. The most recent research with microRNAs and islet culture and pseudoislet culture (organoids) allows predicting advances in knowledge with new drugs to act on the islet/cells (such as the hormone glucagon-like peptide (GLP) -1) as well as induction of other islet cells like alpha-cells and delta-cells to transform into beta-cells.

Key words: Alpha-cell, Beta-cell, Delta-cell, Epsilon-cell, PP-cell

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Introduction

This year 2019 celebrates the 150th anniversary of the description of the pancreatic islet by Paul Langerhans (Berlin, 1847 – Funchal, 1888). In February 1869, Langerhans presented a thesis entitled "Contributions to the microscopic anatomy of the pancreas" (Langerhans, 1869), in which he described the presence of islets of clear cells throughout the gland, with staining properties different from the surrounding tissues. Langerhans noticed that these areas were more richly innervated, but he did not suggest a function, except for the mistaken assumption that they might be lymph nodes (Barach, 1952). However, it was Gustave-Édouard Laguesse (French pathologist and histologist) who named in 1893 the small cellular clusters of the pancreas, the "islets of Langerhans" and postulated that the might act in the digestion. Laguesse also created the term "endocrine" and opened the era of endocrinology with the model: the endocrine pancreatic islet and diabetes (Fossati, 2004). Today, 150 years after the first description, we have accumulated knowledge about the pancreatic islet, its cellular composition, and secretions in physiological situation and disease.

The islets are separated from the surrounding exocrine pancreas by a thin layer of connective tissue intermingled with the connective tissue of the rest of the pancreas. In mature mammals, the islet mass usually corresponds to 1-2% of the mass of the pancreas. The pancreas of the healthy adult human may reach 500,000-1 million islets with a size range of 50-250 μm in diameter (Hellman, 1959b). There is a higher population density of islets in the tail of the pancreas than in the head and body (Hellman, 1959a).

Nowadays, at least five types of cells might be identified in the pancreatic islets responsible for the

secretion of hormones. Therefore, considering the rat islets, we can find the following endocrine cell subsets (Kim et al., 2009):

a. Alpha (α) cells (delivering glucagon, correspond to 20% of islet cells),

b. Beta (β) cells (insulin and amylin – or islet amyloid polypeptide -- are co-secreted in beta-cells in approximately 1:100, the amylin-insulin ratio (Adeghate and Kalasz, 2011), relating to 70% of islet cells),

c. Delta (δ) cells (producing somatostatin, are <10% of islet cells),

d. Epsilon (ε) cells (generating ghrelin, are <1% of islet cells),

e. PP cells (gamma (γ) cells or F cells, supplying the pancreatic polypeptide, are <5% of islet cells),

f. Moreover, serotonin-producing enterochromaffin-cells have been found in the mammalian pancreatic islets at least at some developmental stages (Wierup et al., 2014).

Development of the pancreatic islets

In human, discrete islets can be observed at 12 weeks, and one week later, sizeable primitive islet structures expressing all four pancreatic hormones are formed (Piper et al., 2004). In mice, the first endocrine cells detected in the pancreas express glucagon (Carnegie stage E9.5, 20±1 postovulatory days in human -- Carnegie developmental postsomitic stages 13 to 23 correspond to the second month of life in human) (Mandarim-de-Lacerda, 1987), subsequently, the cells co-express glucagon and insulin, but they are most likely not precursors of beta and alpha-cells. During E13.5, beta-cells are generated in large numbers after extensive growth and branching of the pancreas. Some endocrine cells are also scattered among small aggregates of cells, which suggests that the endocrine cluster is enlarged by the fusion of several small endocrine clusters that originated from different regions of the pancreatic endoderm. This cell cluster is composed primarily of glucagon-expressing cells, with a few randomly distributed insulin-expressing cells (Hara et al., 2007).

This aggregation process, termed "isletogenesis," occurs at E15-E19, with a "ribbon-like" organization of endocrine cells being observed in the central core of the pancreas. This initial phase of islet development occurs during the second trimester, although remodeling occurs throughout late gestation and early childhood (Robb, 1961). Proper islet formation appears to occur through an ensnaring process, which involves the growth of exocrine tissue and leads to a separation of the islets of the "ribbon" to form a "pearls on a string" structure (Jensen, 2004).

Vascular endothelial growth factor (VEGF) is expressed in the developing pancreas or adjacent tissues and promotes beta-cell expansion (Edlund, 2002). Endothelial cells in blood vessels are known to provide inductive signals supporting the development of the pancreas. In the embryo, endocrine pancreatic beta-cells require endothelial signals for their differentiation and function. Mesenchymal tissue rich in a complex capillary network penetrates these immature endocrine cell clusters, which become the islets at 14 weeks gestation (Piper et al., 2004). The concept of a "vascular niche," which is a microenvironment generated by endothelial cells that affect the behavior of adjacent cells, has been applied to beta-cells (Nikolova et al., 2006). Because of intimate interactions between endocrine cells and blood vessels, islets have a highly specialized vasculature with a fenestrated endothelium (Bonner-Weir and Smith, 1994).

At E17, the number of endocrine cell clusters increases, though the mantle-core arrangement of cells does not become more defined, and the beta-cells are in the islet periphery (Leung, 2010). Due to a relatively rapid increase of mature beta-cells, their clusters fuse to form central cores within the endocrine region, and the mature alpha-cell mass then spreads to form a thin mantle layer partially covering the mature beta-cell cores. The arrangement of these endocrine cells within the islet is described as a mantle core, in which a mantle of non-beta-cells surrounds a core of beta-cells with a thickness of one to three cells. This typical arrangement becomes evident at approximately E18-E21 (Kim and Hebrok, 2001; Hara et al., 2007). The formation of islet-like structures occurs earlier in humans and sheep compared to rodents, in which islets do not form until 70% of gestation has taken place (Green et al., 2010).

At the end of embryogenesis and the early postnatal period, high rates of beta-cell proliferation result in the doubling of the number of beta-cells every day in association with vascular growth (Nikolova et al., 2006). Additionally, during isletogenesis, the pancreas will positively stain for both pancreatic and duodenal homeobox 1 (Pdx1) and glucagon (Jensen, 2004). Although in human fetus beta-cells secrete insulin in response to insulin secretagogues (Adam et al., 1969), in rodent beta-cells begin to become responsive to glycemia near term (Kervran et al., 1979). At birth, the cytoarchitecture in the islets is like the adult animal, while their total population size continues to increase. For beta and alpha-cell populations, an additional period of accelerated growth occurs between postnatal days 4 to 10, and growth continues through day 28 due to increased physiological demands of insulin production, most of the islet cells develop within the tail of the pancreas and in the dorsal pancreas (Kaung, 1994; Dhawan et al., 2007).

In addition to proliferation, apoptosis is a necessary process that modulates the development of the endocrine pancreas (Frantz et al., 2012). Beta-cell apoptosis is rare during embryogenesis, although a wave of apoptosis has been described in early postnatal stages, which is thought to be associated with islet remodeling and (or) changes in beta-cell maturation (Scaglia et al., 1997).

The frequency of apoptotic beta-cells in the adult rat is approximately 0.5% and declines progressively until six months postnatal (Scaglia et al., 1997). The

mitochondria play a role in protecting the islets from oxidative stress to prevent apoptosis. Also, insulin-like growth factor (IGF) -II (a survival factor that potentates beta-cell growth, maturation, and functioning and is expressed in beta-cells during the early life of rodents) inhibits the apoptosis of beta-cells (Reusens and Remacle, 2006).

Beta-cell mass expansion in adult continues, and beta-cell hypertrophy in response to increased demand can also contribute to increased beta-cell mass (Bonner-Weir, 2000). However, the regenerative ability of the pancreas, including the islets, decreases with age (Bonal et al., 2008). The regeneration of beta-cells might follow three possible mechanisms: the proliferation of pre-existing beta-cells, neogenesis from an undefined adult progenitor, or stem cells, and transdifferentiation from terminally differentiated cells (particularly in association with conditions such as obesity and pregnancy) (Schwitzgebel et al., 2009). Also, the neuropeptide Y (NPY) receptors may have a role in the control of beta-cell mass. NPY receptor activation functionally protects islets by restoring glucose responsiveness following chemically induced injury. NPY receptor activation attenuates beta-cell apoptosis, preserves functional beta-cell mass and attenuates the hyperglycemic phenotype in a low-dose streptozotocin model of diabetes (Franklin et al., 2018).

There is a variety of sources for the endogenous production of new beta-cells from existing cells. Beta-cells, long thought to be postmitotic, have demonstrated the potential for regenerative capacity. Likewise, the presence of pancreatic facultative endocrine progenitor cells has been established. Also, the malleability of cellular identity has availed the possibility of generating beta-cells from other differentiated cell types (Nichols et al., 2014).

In normal physiological conditions, alpha-cells produce glucagon. In the case of beta-cell damage, alpha-cells also provide glucagon-like peptide-1 (GLP-1 - a growth and survival factor for beta-cells) (Habener and Stanojevic, 2013). In mice, some glucagon-producing pancreatic alpha-cells and somatostatin-producing delta-cells become insulin-expressing cells after the ablation of insulin-secreting beta-cells, thus promoting diabetes recovery. Therefore, islet non-beta-cells (alpha-cells and pancreatic polypeptide-producing gamma-cells), can be reprogrammed by transcription factors to produce and secrete insulin in response to glucose. When transplanted into diabetic mice, converted human alpha-cells reverse diabetes and continue to produce insulin, even after six months (Furuyama et al., 2019). Also, transformed alpha-cells acquire hallmark beta-cell electrophysiology and show glucose-stimulated insulin secretion. The pathways regulated by Aristaless-related homeobox (Arx) and DNA methyltransferase 1 (Dnmt1) are enough for achieving the targeted generation of beta-cells from adult pancreatic alpha-cells (Chakravarthy et al., 2017).

'Programming' is a term used for events during critical developmental windows that interfere with progeny health later in life. 'Fetal programming' corresponds to events in the intrauterine (gestational) period. 'Lactational programming' means events that happen during suckling (until weaning). 'Developmental programming' encompasses the activities during both fetal and lactational lives, while 'postnatal programming' refers to the effect of events either from birth or from weaning (end of lactation) to adolescence (Gusmao-Correia et al., 2012).

These concepts in programming are important because islets are most plastic during early life when programming during fetal and lactational growth is most potent. For example, in mice, the obesity of mothers during gestation and lactation might alter progeny

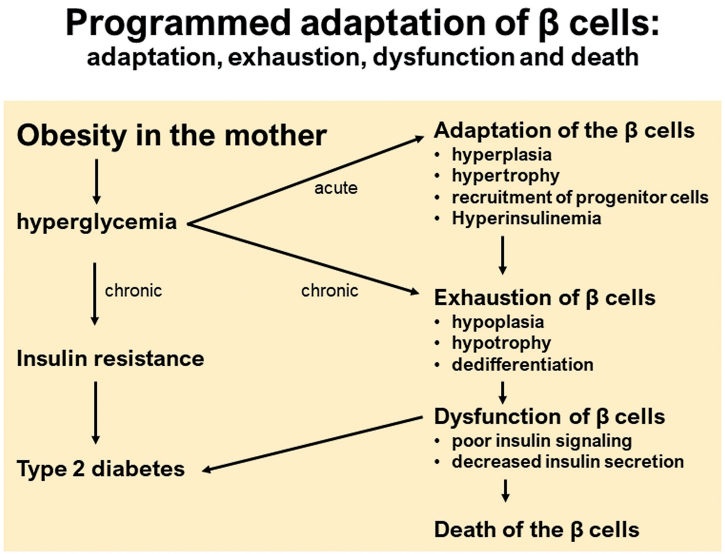

Fig. 1. Adaptation of beta-cells due to prenatal programming (obesity in mother in this example).

metabolism and physiology in adulthood, and chronic hyperglycemia might trigger beta-cell exhaustion, dysfunction, and death (Cerf, 2015) (Fig. 1). Obesity of mothers in mice causes islet structural remodeling in adult male mice offspring coupled with the adverse carbohydrate metabolism and impairment of the insulin-signaling pathway (Bringhenti et al., 2016). Moreover, the protein restriction in mother mice during pregnancy produces structural changes in pancreatic islets of the offspring, with corresponding changes in glucose homeostasis over three consecutive generations (Frantz et al., 2011).

Cytoarchitectural arrangement in the pancreatic islet

Notably, in contrast to the rodent, cell types are much varied in human islets. Rodent islets show a predominant proportion of insulin-producing beta-cells located in the islet core, while the periphery shows a few alpha, delta, and PP cells (Souza-Mello et al., 2011; Abdulreda et al., 2016). However, human islet displays insulin-immunoreactive beta-cells, glucagon-immunoreactive alpha-cells, and somatostatin-containing delta cells scattered throughout the islet (Figs. 2A-B). Also, alpha and beta-cells are in close relationship with each other throughout the cluster (Brissova et al., 2005; Folli et al., 2018).

Human islets contain proportionally fewer beta-cells and more alpha-cells than mouse islets. In human islets, most beta, alpha, and delta cells are aligned along blood vessels with no order or arrangement, indicating that islet microcirculation likely does not determine the order of paracrine interactions (Cabrera et al., 2006; Aamodt and Powers, 2017). In small human islets (40-60 μm in diameter), beta-cells are located in the islet core, while alpha-cells are located peripherally together with vessels. In more prominent islets, alpha-cells have a similar mantle position but are also found along vessels that penetrate and branch inside the islets. The three-dimensional analysis showed islet cells distributed in a trilaminar epithelial plates organization, surrounded by vessels on both sides. In the islet core, the ratio of beta-cells to alpha-cells was higher than in the mantle, decreasing with increasing islet diameter (Fig. 2C) (Bosco et al., 2010). In epithelial plates, beta-cells frequently were disposed in a central position but commonly showed cytoplasmic extensions between outlying non-beta-cells (Baetens et al., 1979).

Circadian clocks working in the endocrine pancreas regulate insulin secretion. Any perturbation in the islet clock in rodents leads to the development of type 2 diabetes (T2D). While whole islet clocks have been studied, the heterogeneity of islet cell oscillators and the interplay between alpha- and beta-cellular clocks for orchestrating glucagon and insulin secretion have only recently gained attention (Petrenko et al., 2018).

Preterm newborns that survive into adulthood show an increased risk for non-communicable diseases, including diabetes. Moreover, chances are increased even for birth at late preterm and early term gestations and for both type 1 diabetes (T1D) and T2D. Thus, factors linked to preterm birth might affect the development of the fetal and neonatal beta-cell in addition to effects on peripheral insulin sensitivity

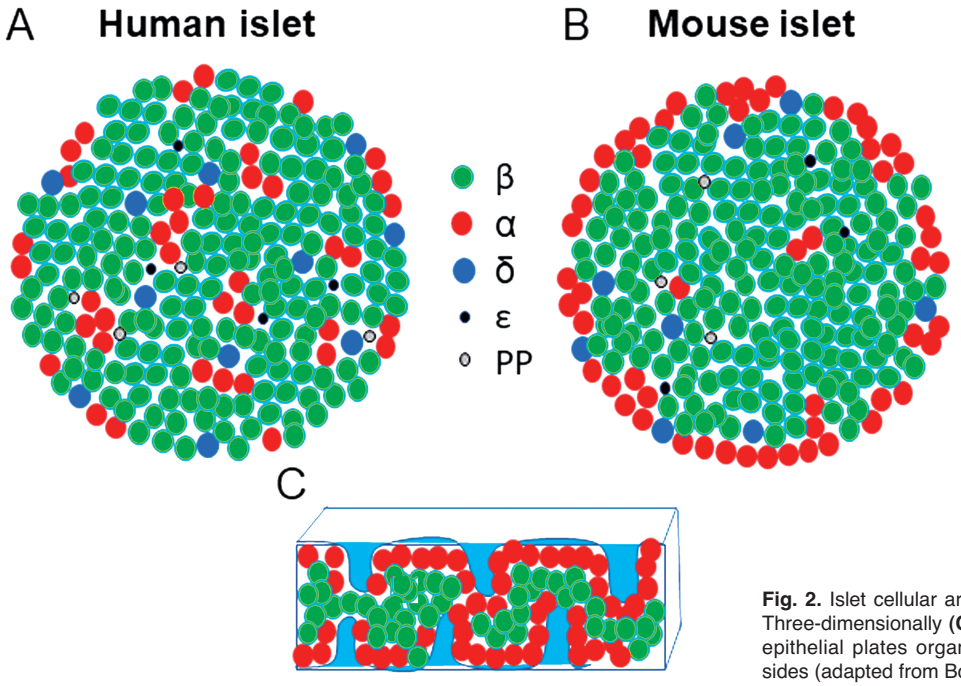

Fig. 2. Islet cellular arrangement in human **(A)** and mouse **(B)**. Three-dimensionally **(C)**, islet cells are distributed in a trilaminar epithelial plates organization, surrounded by vessels on both sides (adapted from Bosco et al., 2010).

(Bloomfield, 2018). Islet inflammation (frequent in T1D) facilitates the access of immune cells to the beta-cells, leading to an irrecuperable and variable extent of beta-cell loss (Morgan and Richardson, 2018). The altered, inflamed islet undergoes a process of infiltrating fibrosis where the island stellate cells have a crucial role (island stellate cells are like the pancreatic stellate cell in phenotype and biological character) (Wang et al., 2018). On the contrary, osteopontin presence may protect pancreatic islets favoring interaction with incretins dependent of Ca^{++} (Cai et al., 2018).

Transcription factors Etv1, Prdm16, Runx1t1, and Bcl11a are essential for pancreas development (Benitez et al., 2014). Moreover, the microRNAs (miRs) like miR152 may have a necessary role in pancreatic beta-cell function (Chen et al., 2018), and miR-302 is involved in *in vitro* dedifferentiation of human pancreatic islet cells and blocks the gene expression related to the maintenance of beta-cell mature phenotype (Sebastiani et al., 2018). In a chimeric graft with a subpopulation of human beta-cells expressing the green fluorescent protein (GFP), both GFP-positive and GFP-negative beta-cells were found within single islets, which demonstrates that beta-cells present in a human islet are derived from multiple progenitors (Scharfmann et al., 2008).

Human beta-cells are not clustered, and most (71%) show associations with other endocrine cells, suggesting unique paracrine interactions in human islets. Also, beta-cells are coupled electrically to six to seven other beta-cells (but not into different cell types). Close cellular proximity and correct anatomical arrangement within islets are essential for standard patterns of insulin secretion, highlighting the functional importance of cell adhesion molecules and connexins (Kelly et al., 2011).

It is noteworthy that vitamin D deficiency in obese mice (presenting hyperinsulinemia, hyperleptinemia, and insulin resistance), leads to an enlargement of the islet, including alpha and beta-cell disarray (Borges et al., 2016). However, correlations between body mass index, pancreas weight, and beta-cell/islet mass are low or not significant (Dybala et al., 2019).

Somatostatin is secreted by islet delta-cells and by extra-islet neuroendocrine cells. Also, on alpha- and beta-cells somatostatin receptors have been identified, and exogenous somatostatin might regulate alpha- and beta-cell function, blocking insulin and glucagon secretion. Therefore, delta cells exert a tonic inhibitory influence on insulin and glucagon secretion (Hauge-Evans et al., 2009). The activation of somatostatin receptors coupled to the inhibitory G protein mediates the effects of somatostatin culminating in the suppression of the electrical activity and exocytosis in alpha- and beta-cells (Rorsman and Huising, 2018). Also, somatostatin cells in the neonatal mouse play a critical role in the control of insulin release and normal islet function and are essential for neonatal survival and the maintenance of glucose homeostasis (Li et al., 2018).

Ghrelin is a peptide hormone predominantly produced in the stomach, but also other tissues, including the pancreatic islet (epsilon cells). Ghrelin is an insulinostatic hormone, and ghrelin-expressing pancreatic tumors have been reported (Wierup et al., 2014). The epsilon cells are most numerous pre- and neonatally and, in humans, constitute 10% of all islet cells from mid-gestation to birth (Frantz et al., 2012). Since gastric ghrelin expression is low before birth, the islets may be the primary source of circulating ghrelin during this time. The secretory granules of epsilon cells are of small size with a mean dense-core diameter of 110 nm (Wierup and Sundler, 2005).

In healthy subjects, PP-cells (or gamma (γ) cells or F cells) appear sex-related, and male individuals show a significantly higher density of PP-cells than female (Stefan et al., 1982). A PP-cell rich region is found mainly in the uncinate process, and the distinct distribution of PP-cells includes irregularly shaped clusters composed solely of PP-cells. Furthermore, in the PP-cell rich region, beta- and alpha-cell mass is significantly reduced compared to surrounding PP-cell poor areas (Wang et al., 2013).

Amylin and neurodegenerative disease

Amylin is a 37-amino acid peptide hormone co-stored and co-secreted with insulin by pancreatic islet beta-cells. Amylin inhibits food intake, delays gastric emptying, and decreases glycemia (Bower and Hay, 2016). The conformational alpha-helix monomers of amylin are responsible for its physiological actions. The misfolding unstable oligomers may be detrimental to the islet beta-cells, leading to a beta-sheet fibrils transformation as amyloid deposits. Although no direct evidence proves that the amylin fibrils in amyloid deposits cause diabetes, human amylin may modulate autoimmunity and inflammation through regulatory T cells, impacting on both human T1D and T2D (Zhang et al., 2016).

Human amylin aggregates and human amylin oligomers might disrupt the membrane of islet beta-cells and cause endoplasmic reticulum stress, and mitochondrial damage (Atsmon-Raz and Miller, 2016), and human amylin oligomers also inhibit autophagy, although autophagy can function to remove amylin aggregates. Therefore, it is crucial the protection of beta-cells from cytotoxic amylin (Kiriyama and Nochi, 2018). Islet amylin deposits are found in pancreatic islets of more than 90% of T2D patients, and an association between amylin aggregation and reduction in beta-cell mass was observed in post-mortem studies (Azzam et al., 2018).

In metabolic disturbances, mainly T2D might increase the risk of cognitive decline and Alzheimer's disease (is it a type 3 diabetes?) (Alzheimer research forum live discussion: Is Alzheimer's a type 3 diabetes?, 2006; Pruzin et al., 2018). Furthermore, both Alzheimer's disease and T2D are amyloidogenic diseases, with the abnormal aggregation of amyloid-beta

peptide and amylin, respectively contributing to cellular death and disease pathogenesis, and amyloid-beta peptide may have peripheral effects including its co-deposition in the pancreas (Wijesekara et al., 2018). T2D and Alzheimer's disease are both highly prevalent diseases worldwide, and each is associated with high-morbidity and high-mortality. A mechanism includes islet amyloid polypeptide (or amylin) deposition, co-localized with beta-amyloid and found in more abundance in the Alzheimer's disease temporal cortex (Pruzin et al., 2018).

Pancreatic islets: research and transplantation

A visible indicator of the health of the endocrine pancreas would be the determination of the number of beta cells in the organ. This number, however, has a high operational 'cost' to be defined, even in experimental studies (Bock et al., 1999). The method for estimating the number of beta cells in the pancreas should employ the use of stereological techniques, organ fractionation and a strategy known as 'disector' (Mandarim-de-Lacerda, 2003; Mandarim-de-Lacerda and Del Sol, 2017).

A 'less costly' alternative to evaluate the health of the endocrine pancreas, or to evaluate the evolution of insulin resistance to TD2, could be the measure of beta cell mass. The total mass of beta-cells is a critical factor in the regulation of glucose homeostasis and islet morphology (Inuwa and El Mardi, 2005) and results from a balance between the dynamic changes of new cell growth and old cell loss (Bonner-Weir, 2000). Indeed, the reduction of beta-cell mass means a poorly evolving carbohydrate metabolism disorder.

In experimental studies, we have proposed a feasible method for the estimation of beta cell mass that allows the comparison of experimental groups ranging from the provision of different diets (Frantz et al., 2011) to the use of drugs (Souza-Mello et al., 2011). Briefly, the pancreas must be weighed and thoroughly sectioned. A significant sample of pancreatic sections, stained with hematoxylin and eosin, will be used for identification and quantification of the islets. Another sample of sections, immunolabelled with anti-glucagon antibody and anti-insulin antibody, will allow the identification of the alpha-cells and beta-cells. It is then possible, to establish which fraction of the pancreas is occupied by the islets, called the volume density of the islets (Vv [islet, pancreas]). The simplest way to do this estimation is by 'point counting.' On the stained sections of the pancreas (which contain the islets) we put a test system consisting of regularly spaced points within a known area frame. Vv [islet, pancreas] is estimated as the ratio of the number of points that hit the islets (Pp) and the total number of points in the system (PT) (Mandarim-de-Lacerda and Del Sol, 2017). The mass of islets (M [islet]) can be estimated by the ratio of the mass of the pancreas to Vv [islet, pancreas].

Pancreas sections containing islets that have not been used so far should follow the immunostaining of alpha-cells and beta-cells. The volume densities of the alpha-cells (Vv [alpha-cells]) and beta-cells (Vv [beta-cells]) should be estimated by image analysis (Mandarim-de-Lacerda et al., 2010). Thus, the density threshold selection tool of the image analysis facility should be used on islets with glucagon-positive areas and insulin-positive areas. Vv [alpha-cells] and Vv [beta-cells] are expressed as a percentage of the islets. Alpha-cell mass (M [alpha-cells]) and beta-cell mass (M [beta-cells]) might then be estimated as the product of [Vv [alpha-cells] or Vv [beta-cells], and M [islet] (Frantz et al., 2011)

Pancreatic islets from different species may function in a particular way. Therefore, it is prudent to consider the features of the species before optimizing islet isolation protocols (Abdulreda et al., 2016). *In vitro* studies with cultured islet, beta-cells (monolayer) or islet-like clusters (pseudoislets or organoids) are frequently used to test factors or drugs. Islet and pseudoislets have the advantage of maintaining the capacity to form an intact islet-specific microvasculature (angioarchitecture), which appears to be independent of the cellular composition of pseudoislets (Vajkoczy et al., 1995; Candiello et al., 2018). The microphysiological analysis platform (MAP) allows the 3D spheroid formation of pancreatic beta-cell islets, large-scale morphological phenotyping, and gene expression mapping of chronic glycemia and lipidemia development. The beta-cells' MAP might provide a potential new map in the pathophysiological mechanisms of beta-cells (Lee et al., 2018).

Pancreas allotransplantation requires immuno-suppression, a risk that should be considered before the procedure. Pancreas transplantation has associated morbidity, although it is linked with an improvement in diabetic micro- and macro-angiopathy. Simultaneous pancreas-kidney and pancreas after kidney transplantations should be proposed for kidney recipients with T1D with no surgical, especially cardiovascular, contraindications (Wojtusciszyn et al., 2019).

The minimally invasive radiological or mini-surgical procedure of islet transplantation consists of the infusion of purified islets via the hepatic portal vein (frequently it needs to be repeated two or three times to achieve insulin independence and long-term functionality) (Anazawa et al., 2019). The method for automated isolation of human pancreatic islets has existed since the late '80s (Ricordi et al., 1988). Recent progress in techniques for islet isolation, islet culture, and peri-transplant management of the islet transplant recipient has resulted in substantial improvements in metabolic and safety outcomes for patients (Rickels and Robertson, 2018). With the development of alternative transplantation sites and new cell sources, including porcine islet cells and embryonic stem/induced pluripotent stem-derived beta-cells, one might consider an "On-demand," and "Unlimited" cell therapy for T1D (Anazawa et al., 2019).

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