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Review

Treatment of Canine Type 1 Diabetes Mellitus: The Long Road from Twice Daily Insulin Injection towards Long-Lasting Cell-Based Therapy

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Abstract: For over 150 years, researchers have studied the (patho)physiology of the endocrine pancreas and devised treatment options for diabetes mellitus (DM). However, no cure has been developed so far. In dogs, diabetes mellitus type 1 (T1DM) is the most common presentation. Treatment consists of twice daily insulin injections, monitored by spatial blood glucose measurements. Even though dogs were instrumental in the discovery of insulin and islet transplantations, the treatment in diabetic dogs has remained unchanged for decades. Providing twice daily insulin injections is demanding for both owners and dogs and may result in hypoglycaemic events, creating the need for new treatment strategies. Novel regenerative medicine-based tools, such as improved β-cell culture protocols and artificial devices, have sparked hope for a cure. In human medicine, emerging technologies such as the transplantation of insulin-producing β-cells, generated by stem cell differentiation, with or without an encapsulation device, are currently tested in phase I/II clinical trials. As the pathogenesis of T1DM is remarkably similar between humans and dogs, novel treatment methods could be implemented in canine medicine. This review briefly summarises the physiology of the canine endocrine pancreas and the pathophysiology of canine DM before exploring current and possible future treatment options for canine DM.

Keywords: dogs; insulin; pancreas; organoids; regenerative medicine

Citation: Oliveira, F.C.M.; Voorbij, A.W.Y.; Pereira, E.C.; Alves e Almeida, L.M.M.; Moraes, G.R.; De Oliveira, J.T.; Gouw, B.H.T.; Legatti, S.A.M.; Kooistra, H.S.; Spee, B.; et al. Treatment of Canine Type 1 Diabetes Mellitus: The Long Road from Twice Daily Insulin Injection towards Long-Lasting Cell-Based Therapy. *Organoids* **2024**, *3*, 67–82. https://doi.org/10.3390/organoids3020006

Academic Editor: Süleyman Ergün

Received: 21 December 2023
Revised: 14 February 2024
Accepted: 28 March 2024
Published: 4 April 2024

1. Introduction

The endocrine pancreas was first studied in 1868 by Paul Langerhans [1]. By performing a total pancreatomy in a dog, Minkowski demonstrated the important role of the pancreas in the aetiology of diabetes mellitus (DM), as its absence resulted in hyperglycaemia and glucosuria [2]. Almost 50 years later, Banting and Best isolated insulin from dogs and provided proof for insulin therapy used as the treatment for DM [3]. Despite extensive molecular, cellular, and clinical research ever since, no cure for DM was found and the disease is still treated symptomatically. Insulin therapy has a high impact on the life of patients with DM and may be associated with complications such as hypoglycaemia, highlighting the clear need to develop better treatment strategies.

In this article, a brief overview of the organogenesis and physiology of the endocrine pancreas, the pathophysiology of canine DM, and the current and future treatment methods of canine type 1 diabetes mellitus (T1DM) is provided. The emphasis will lie on the potential

treatment options based on recent advancements in the field of regenerative medicine, such as β-cell cultures. For excellent reviews on the genetic background of canine DM, the readers are referred elsewhere [4,5].

2. Diabetes Mellitus

According to the World Health Organization (WHO), DM is a metabolic disorder of multiple aetiologies characterised by persistent hyperglycaemia with disturbances of carbohydrate, fat, and protein metabolism, resulting from a defect in insulin secretion, insulin action, or both [6]. The WHO's current diabetes classification comprises two main categories: type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). While T1DM in humans is related to idiopathic immune-mediated destruction of β-cells, T2DM is of multifactorial origin and is characterised by relative insulin resistance and/or inadequate insulin production. Other types of DM include gestational DM and drug-induced DM. In both humans and dogs, T1DM is characterised by selective destruction of β-cells without the presence of inflammatory cells on histopathology [7,8]. Due to these similarities, dogs suffering from T1DM can benefit from the discoveries done in humans. This is in sharp contrast to the early days of diabetes research, where dogs were exposed to, maybe nowadays considered as unethical, experiments to aid human medicine.

The diagnosis of T1DM in dogs is based on clinical findings, persistent hyperglycaemia, and glucosuria [9]. Reporting an overall incidence of around 3% [10–12] and a hospital prevalence rate of 0.4–1.2% in dogs and cats, diabetes is considered a common disease in companion animals [13].

Due to the lack of functional β-cells in dogs with T1DM, there is a decreased or absent response to hyperglycaemia in insulin production leading to clinical signs such as polyuria, polydipsia, polyphagia, and glucosuria when blood glucose concentrations rise above 11.0 mmol/L [9,14,15]. Other clinical signs may be weakness, depression, vomiting, dehydration, gait abnormalities, and cataract formation [9]. Diabetic ketoacidosis and hypoglycaemia (resulting from its treatment) are regarded as life-threatening conditions [14,16,17]. A survey revealed that within two years after diagnosis, 20% of the owners opted for euthanasia, of which 50% already decided to euthanise their pet at the time of diagnosis [18]. Although the motivations for euthanasia differed, costs, pet welfare, and impact on the owner's life were frequently mentioned (32–44%), as well as concurrent disease (±45% of respondents). This survey, with over one thousand veterinarians responding, clearly highlights the importance of finding more sustainable treatment options.

3. Current Treatment of Canine Type 1 Diabetes

The current treatment of canine T1DM does not provide a cure but aims to manage its symptoms and avoid ketoacidosis and hypoglycaemia [9]. This is performed by twice daily subcutaneous insulin injections, to maintain the blood glucose concentration within an acceptable range [13]. It is a life-long treatment that requires tremendous motivation and discipline from the dog's owner(s) [15]. In 2018, the guidelines of the American Animal Hospital Association (AAHA) stated that T1DM dogs should be treated with exogenous insulin [9]. After establishment of the correct insulin dosage, the dog is treated and monitored through observation of clinical signs and by measuring blood glucose levels at specific time points [9,15]. Additionally, serum fructosamine (glycated albumin) and glycated haemoglobin levels can be used to monitor treatment results [19–21].

Dietary changes can also assist in the control of T1DM. A high fibre diet with soluble fibres [22] and a combination of insoluble and soluble fibres are associated with lower postprandial plasma glucose concentrations and reduced plasma fructosamine levels [23]. This prompted the pet food industry to produce specific dog foods aiming to control post-prandial plasma glucose concentration.

4. Current Treatment Failures

Even though a portable glucometer seems like a convenient and advantageous tool to regulate blood glucose concentrations in dogs, some owners have difficulties obtaining blood samples from their pets, which could result in treatment errors [24,25]. Another option to evaluate blood glucose concentrations is the use of a continuous glucose monitoring (CGM) system. However, these systems measure the glucose concentration in the interstitial fluid, not in blood. Therefore, rapid changes in blood glucose levels are not immediately detected with these systems. Due to this delay of about 10 min, hypoglycaemia could be missed [26]. Hypoglycaemia can be life-threatening for T1DM dogs, since it can occur without overt clinical signs [27,28].

5. Organogenesis and Physiology of the Endocrine Pancreas

To create a safe, functional, and long-lasting cell-based alternative for the cumbersome twice daily injections of insulin combined with frequent glucose monitoring, integrating knowledge from pancreatic organogenesis and physiology is of utmost importance.

The organogenesis of the mammalian pancreas is a highly complex and broadly understood process and still an active field of research [29–31]. For excellent reviews, readers are referred elsewhere [32–34].

The pancreas comprises exocrine and endocrine compartments (Figure 1). The acinar and ductal cells, which make up 95% of the organ's cell mass, form the exocrine part of the pancreas, which relates to digestion. Acinar cells are responsible for the synthesis, storage, and release of digestive enzymes (amylase, lipase, etc.) into the duodenum via the pancreatic duct [35,36]. The endocrine pancreas, embedded in the exocrine pancreatic tissue, consists of the islets of Langerhans. Its main function is the management of the organism's metabolism by the release of hormones into the bloodstream. Five different endocrine cell types can be found within the islets, which originate from multipotent pancreatic progenitor cells: α-, β-, δ-, ε-, and pancreatic polypeptide cells (PP-cells) (reviewed in [37]). There are differences in the relative cell contents and localisation of the diverse cell types among various mammals [38]. The canine pancreas consists of slightly over 50% of β-cells mainly located in the core, slightly lower than 30% of peripherally located α-cells, and a low number of δ-, ε-, and PP-cells [38]. Human islets' cytoarchitecture resembles it to a certain degree [38], where α-cells (36%) and β-cells (54%) are located both in the core and periphery (36%) [39,40]. The relative amount of the various cell types has recently been confirmed by single cell RNA technology [41–43].

The blood glucose concentration is not solely regulated by insulin; other hormones produced by endocrine cells aid in this process. Glucagon, secreted by the α-cells, is the counteracting hormone to insulin [44]. The main target cell of glucagon is the hepatocyte, where it stimulates glycogenolysis and gluconeogenesis and therefore increases the blood glucose concentration [45]. Somatostatin, a hormone released by the δ-cells, inhibits the secretion of insulin, glucagon, and pancreatic polypeptides [46]. PP-cells secrete pancreatic polypeptides under low-glucose conditions that inhibit glucagon release from α-cells [47], and ε-cells secrete ghrelin, a hormone that suppresses insulin release from β-cells [48].

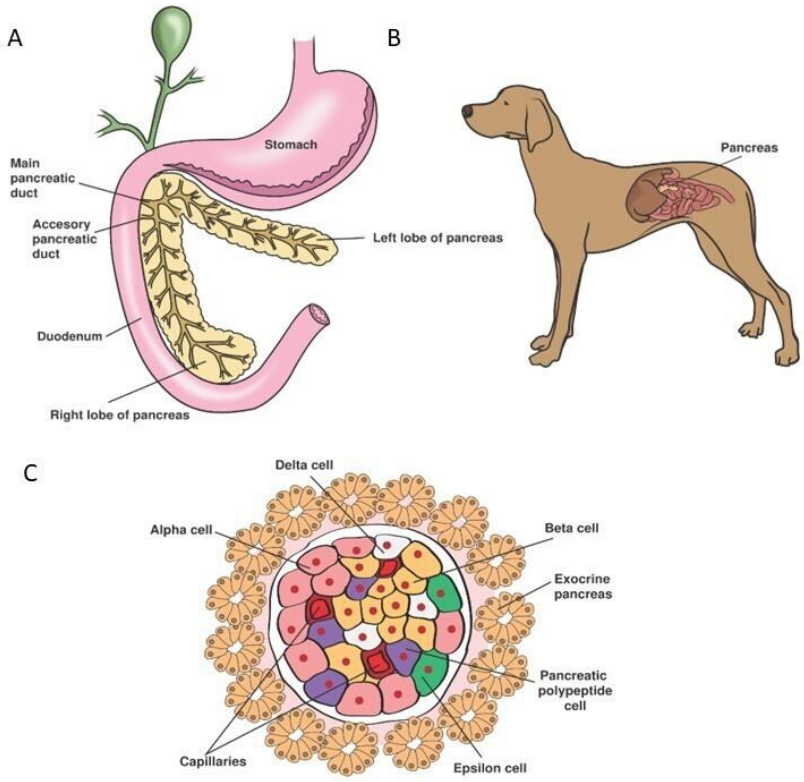


Figure 1. (**A**) Anatomy of the canine pancreas: a slender, V-shaped organ situated between the greater curvature of the stomach and the descending duodenum. (**B**) The canine pancreas in situ. (**C**) An architectural model of the canine pancreas: the exocrine pancreas surrounds the islet of Langerhans (endocrine pancreas). Figure created in Adobe Illustrator 2020 (Adobe, San Jose, CA, USA).

In recent years, new approaches to the treatment and cure of different diseases became possible with emerging regenerative medicine techniques, such as stem cell technology and organoid cultures. While pancreas and islet allotransplantations are limited by the number of available donors, stem cell therapy comes with the promise of an infinite (autologous) source of cells to culture, that can be differentiated towards insulin-producing cells (IPCs) that could be transplanted into the patient and thereby provide a cure for T1DM. Three different types of stem cells can be considered for this purpose: pluripotent embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and adult stem cells (ASCs) that have a more restricted differentiation potential [49,50]. When exposed to specific growth factors that for instance mimic embryonic development and embedded in a suitable extracellular matrix, these cells can form long-term cultures in vitro resembling the organ-related characteristics in vivo, called organoids [50]. Organoids bridge between in vitro and in vivo models, evaluating biological cell functions, drug screening, and cellular differentiation and in addition might be used for cell transplantations (Figure 2). Organoid technology is gaining momentum in veterinary research as indicated by studies performed on organoids in dogs and cats with various organs, like the liver [51–54], intestine [55–57], and skin [58]. Direct reprogramming of stem cells (skin-derived?) is attractive. The advantage of adult stem cell-derived organoids is in the fact that this can lead to a mini-organs with various cell types that are present in the islets of Langerhans in vivo, not purely β-cell composition. Thus far, our own experience shows the main cellular constituents of adult stem cell-derived canine organoids are ductular cells, with only very few glucose-responsive β-cells (unpublished data).

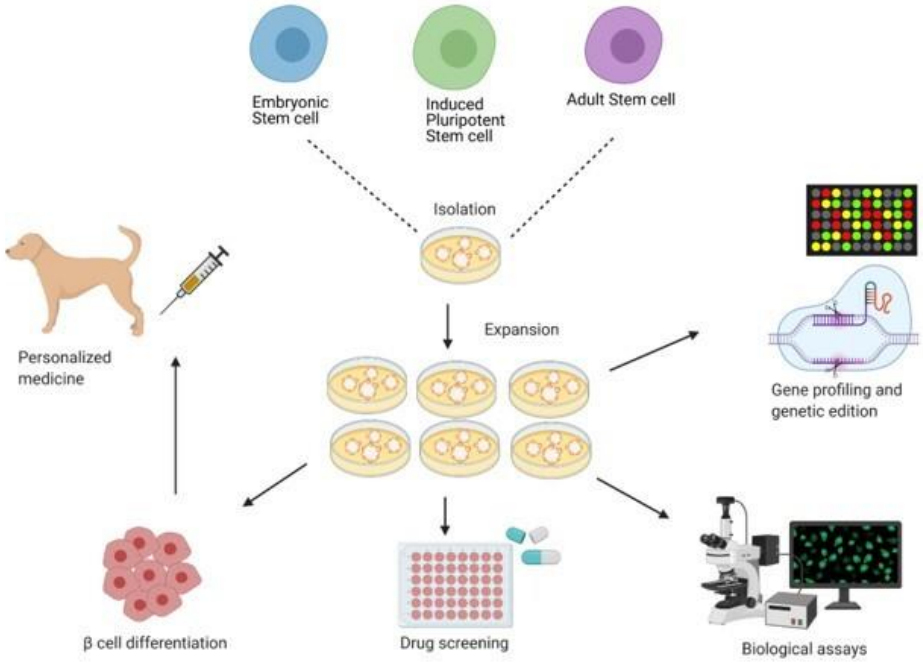


Figure 2. Novel applications of organoid technologies from different cell sources. Pluripotent stem cells (embryonic stem cells and induced pluripotent stem cells) and adult stem cells can be harvested, isolated, and expanded long-term in vitro. One of the most desirable goals of regenerative medicine in type 1 diabetes mellitus (T1DM) patients is replacement of β-cells by cells produced in vitro. Drug screening, biological assays, gene profiling, and gene editing are important tools to study the pathophysiology of T1DM and may result in clinical applications. Figure created in Biorender.com.

6. Future Treatment of Canine Type 1 Diabetes

Bioengineers can propose alternative ways to overcome the lack of functional β-cells in T1DM patients through medical and cellular devices.

6.1. Insulin Implantable Pumps and Continuous Glucose Monitoring Systems

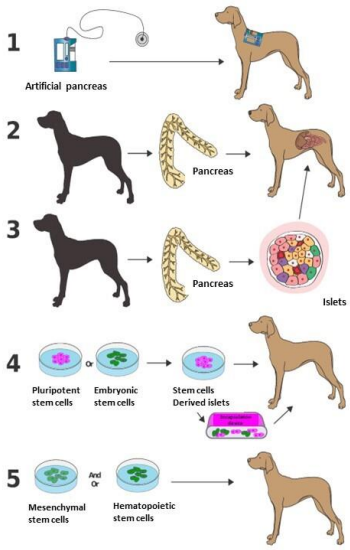
There are CGM systems on the market for use in dogs, which could aid in T1DM management. These devices are a useful tool to support the optimisation of blood glucose concentrations in canine DM patients, which improves the quality of life of dogs and owners [59–62]. However, rapid changes in blood glucose are not automatically followed by insulin release and are not immediately reflected by similar changes in the extracellular glucose concentration but follow with some delay. Therefore, even correct use of these systems can still lead to threatening conditions such as severe hypoglycaemia if the insulin levels are not adequately adjusted [59].

Studies using an insulin implantable pump have not been described in privately owned dogs but have been performed in experimental dogs as a translational animal model [63]. In this study, the pump delivered insulin intraperitoneally for 28 months without presenting problems and maintained glycaemic levels within a certain range (8.3–16.7 mmol/L) in more than 65% of the obtained blood glucose samples [63]. No hypoglycaemic coma, infection, or skin irritation was described. The insulin implantable pump represented a simple, stable, and safe device for dogs.

6.2. The Artificial Pancreas

The artificial pancreas combines an implantable insulin pump, a CGM system, and a control algorithm that can turn the insulin administration on and off, based on sequential glucose measurements throughout the day [64]. In 1982, in search of an accurate and small enough device to treat human DM patients, Shichiri et al. placed a wearable artificial endocrine pancreas on three pancreatectomised dogs. Based on a closed-loop control

system, this model restored blood glucose levels for up to seven days. Unfortunately, it had a very short lifespan and gradually lost its sensitivity and effectivity [65]. Since then, the dog has been used extensively as an animal model for the realisation and optimisation of the human artificial pancreas [66–69]. However, recently no studies have been reported that evaluated an artificial pancreas for the treatment of diabetes mellitus in dogs (Figure 3(1)).



	PROS	CONS
• Artificial pancreas	• Lifesaving • Insulin injections programmed • Simple, stable	• Expensive • Life-long treatment
• Pancreas transplant or Islet cells transplant	• Curative treatment, if no further immune destruction of β-cells • Improved glycaemic control • Relativelly safe • Cellular source raises	• Expensive • Acute rejection risk • Infection risk • Extreme ethical issues • Scarcity of donors • Thecnical expertise • Logistic issues • Use of immunosuppressive drugs
• Stem cells technology	• Easily accessible cellular source • Autologous transplant • No immune risk • Unlimited source of insulin-producing cells	• Very expensive • Extreme ethical issues • Logistic issues • Technical expertise • Formation of teratomas • Improvements required to vascularisation • Biocompatibility of devices • Bioengeneering solutions to avoid autoimmune response • Good manufacturing practice (GMP)

Figure 3. Possible future treatments of canine type 1 diabetes mellitus: (**1**) an artificial pancreas placed on a dog. (**2**) The allotransplantation of a pancreas from a donor dog to a diabetic dog. (**3**) The allotransplantation of pancreatic islets from a donor dog to a diabetic dog. (**4**) Differentiation of pluripotent stem cells or embryonic stem cells into β-cells that are transplanted with or without an encapsulation device to the diabetic dog. (**5**) The injection of mesenchymal stem cells and/or hematopoietic stem cells for immunomodulation in favour of β-cell survival. In parallel, the pros and cons of each approach are informed. Figure created in Adobe Illustrator 2020 (Adobe, San Jose, CA, USA).

6.3. Pancreatic or Islet Allotransplantations

Pancreatic or islet transplantations have a long history in human patients that evolved in parallel with experimental procedures in mice and dogs (reviewed in [70,71]). At that time, the aim was not to treat diabetic dogs, but to use those dogs as an animal model to perform pancreatic or islet transplants on that could generate knowledge to be applied in human medicine. The first successful canine pancreas allotransplantation was reported in 1927 [72] and continued ever since [73–78] (Figure 3(2)). Unfortunately, long-term use of immunosuppressive drugs remains essential in these patients (reviewed in [79]). One of the technical bottlenecks in pancreas allotransplantation is the surgical risk that digestive enzymes of the exocrine pancreas leak into the abdominal cavity during the procedure, resulting in significant morbidity and/or mortality [71]. Therefore, research has focused on the development of islet transplantation techniques as a less invasive alternative to replace the non-functional pancreas (Figure 3(3)). Islet dissociation, purification, and possible ectopic sites for transplantation (intraperitoneal, intramuscular, subcutaneous, and intrahepatic) were investigated in rodents, pigs, and dogs [70], and the liver is now considered the gold standard for ectopic transplantation sites in rodents [80].

Dogs were used as a pre-clinical large animal model before islet cell transplantations were performed in humans. In most studies, pancreatic islet allotransplantation was conducted in healthy dogs that were made insulin-dependent by either pancreatectomy or drug therapy [81–85]. However, a few studies were performed in dogs with spontaneous DM (Table 1).

Table 1. A summary of studies conducting pancreatic islet transplantation in dogs with spontaneous diabetes mellitus.

Source	Year	Procedure	Encapsulation Method	Outcome
Alejandro et al. [86]	1985	Packed islet cell volume of 1.5 mL or less. Immunosuppressive agent: cyclosporin.	Implantation in the liver.	Normoglycaemia for at least 30 days. In the absence of cyclosporin: allograft rejection within a range of 4–10 days.
Alejandro et al. [87]	1988	Multi-donor intrahepatic islet allografts. Immunosuppressive agent: cyclosporin.	Implantation in the liver.	Six dogs maintained normoglycaemia between 253 and 716 days.
Brunetti et al. [88]	1991	Enveloped porcine islets. No immunosuppression.	Microspheres in an artificial vascular prosthesis.	For 60 days, one dog reached complete insulin-independence. The others presented blood glucose levels near normal range.
Lanza et al. [89]	1992	Encapsulated canine islets.	Intraperitoneal implantation in rats.	Normoglycaemia for 1 month.
Lanza et al. [90]	1995	Encapsulated systems.	Intraperitoneal implantation.	Normoglycaemia mantained from 90–365 days.
Soon-Shiong et al. [91]	1992	Microencapsulated islets. Cyclosporin in subtherapeutic dose.	Intraperitoneal injection.	All dogs maintained normoglycaemia for 63 to 172 days.
Lanza et al. [92]	1999	Alginate gel microencapsulated islets. Low dose of cyclosporin.	Implantation into the peritoneum.	All dogs maintained normoglycaemia for 60 to 175 days.
Abalovich et al. [93]	2009	Polylysin-alginate microencapsulated porcine islets. No immunosuppression.	Implantation in the abdominal cavity.	Decrease in the need for exogenous insulin and higher plasma insulin levels.

In 1985, the first allotransplantation of at least 30×10^3 islets in the liver of dogs with spontaneously occurring T1DM as well as in pancreatectomised dogs was reported, which resulted in normal blood glucose levels [86]. The dogs were treated with cyclosporin-A as the immunosuppressant. After removal of this drug (30-, 60-, or 90-days post-transplant), the transplanted cells were gradually rejected. Three years later, the same research group transplanted multi-donor intrahepatic islet allografts into eight dogs with spontaneous T1DM [87]. To achieve immunosuppression, cyclosporin-A was used. Six of these dogs maintained normoglycaemia between 253 and 716 days.

Allotransplantation of islets of Langerhans in an encapsulated system emerged as an alternative to avoid the need for immunosuppressive drugs due to their ability to keep these islets out of reach of the patient's immune system. In 1991, Brunetti et al. made enveloped porcine islets and transplanted these into five dogs with T1DM [88]. One of the dogs reached complete insulin-independence after transplantation, whilst the others presented near-normal blood glucose levels. In 1992, Lanza et al. transplanted encapsulated canine islets intraperitoneally in diabetic rats using a permselective cylindrical chamber. The blood glucose levels decreased within 24 h and normoglycaemia was maintained for at least one month [89]. In 1995, the same authors studied three different types of encapsulation systems implanted intraperitoneally without the use of immunosuppressive drugs. Canine islets were introduced in a chamber surrounded by a permselective acrylic membrane or in alginate sphere microreactors. Both devices resulted in improved glucose homeostasis ranging from three months duration to one year, which was the latest time point in this study design [90]. Despite the long-term use of these immune-isolated islets,

some issues remained to be explored, such as the biocompatibility of the materials used and the appropriate implantation site.

The use of a microencapsulation system in dogs was first studied by Soon-Shiong et al. in 1992 [91] (Figure 3(4)). After intraperitoneal transplantation of alginate encapsulated islets in seven dogs with spontaneous DM, normoglycaemia was maintained for 63 to 172 days. In 1999, Lanza et al. conducted a similar study; the islets were encapsulated inside of an alginate gel and transplanted into the peritoneum of four dogs with spontaneous DM [92]. The results were remarkably alike; all four dogs maintained their insulin-independence for 60 to over 175 days. In 2009, Abalovich et al. performed islet transplantation in five diabetic dogs using microencapsulated porcine pancreatic islets. The blood glucose concentrations, plasma insulin levels, and glycosylated haemoglobin results 6 to 12 months after the procedure were significantly improved [93].

Despite the development of encapsulation technology of islet cells in dogs, the need for donor pancreas procurement, technical expertise, infrastructure, good manufacturing practice (GMP) conditions, ethical concerns, complex coordination, and high costs still hamper its implementation in the veterinary clinic [15,94]. Although Yang et al. developed a specific protocol for canine islet isolation and transplantation [85] and despite recent efforts to establish a canine pancreas donor programme [95], no steps have been taken towards the implementation of this therapy as a commercial treatment option for dogs with T1DM. The lack of translation of these preclinical studies into veterinary practice remains remarkable. This is possibly caused by the logistical and ethical issues related to the source of the donor pancreas. How easily are brain-dead or fresh cadavers available in a clinical setting that can be used to perform these transplantations? The obvious ethical issue related to cure a diabetic dog while sacrificing a donor dog is a no-brainer. In view of the recent progress in the development of humanised pigs, from which the donated organs do not elicit an immune reaction in men, it seems possible to create caninised pigs. This strategy in itself has specific ethical issues related to the status of the donor animal.

6.4. Stem Cell Approach to the Treatment and Cure of T1DM

Because the loss of β-cells is the basis for insulin deficiency in T1DM, one of the most desirable goals in regenerative medicine is replacement of this specific cell type by cells produced in vitro. To date, fully differentiated pancreatic β-cells generated in vitro have been produced only at a low efficiency rate, so further improvements in cell functionality and maturation are of utmost importance [96–98]. For this reason, the sequential transcription factors that are expressed and silenced during pancreatic organogenesis have been studied in animal models (mainly rodents) and were used to create protocols for the controlled differentiation of endocrine cells from stem cells [99]. The challenge to obtain functional β-like cells from pluripotent stem cells (PSCs,) was finally overcome in 2014 by Pagliuca et al. [100]. During pancreatic organogenesis, pancreatic and duodenal homeobox 1 (PDX1), NK6 homeobox transcription factor-related locus 1 (NKX6.1) and Sry-related HMG-box gene 9 (SOX9) are the key transcription factors that regulate pancreatic development [101,102]. In this embryogenic process, in which the inhibition of Notch signalling pathway is crucial, Neurogenin-3 (NGN3) is transiently expressed to initiate the specification of all endocrine cell types (α-, β-, δ-, ε- and PP-cells) [33]. In humans, the role of NGN3 in the endocrine fate also seems important in pancreatic development, however it is unknown which signalling- or niche-factor is decisive in directing the differentiation of a specific endocrine cell lineage. In the last stages of differentiation protocols, the expressions of V-maf avian musculoaponeurotic fibrosarcoma oncogene homolog A (MAFA) and NKX2.2 markers play a role in the activation of insulin gene expression and therefore in its maturation and functionality [101].

Although organogenesis is very similar between mammalian species, some adjustments need to be made in the various culture conditions of adult stem cells. This process is mainly a matter of trial-and-error, unfortunately [99]. Some spatiotemporal differences in organogenesis are reflected in the time point at which different markers are expressed.

NKX2.2 in mice, for example, is expressed in multipotent pancreatic progenitors, while in humans, it is a marker of endocrine commitment, detected after NGN3 transient expression [33]. There is little information about β-cell organogenesis in dogs, but in 2013, Takemitsu et al. showed that, similar to mice and humans, PDX1 and MAFA markers play a role in insulin mRNA expression in dogs [103].

6.4.1. Embryonic Stem Cell and Induced Pluripotent Stem Cell

Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are both pluripotent cells, which can be used to generate β-cells [50]. Because ESCs can only be obtained by taking the inner cell mass from a blastocyst [104], its use imposes different logistical and ethical problems. However, because iPSCs are made from the patient's own cells, it can be used for autologous transplantation [49].

The first protocol differentiating human ESCs into definitive endoderm was described in 2005 [105]. Since then, many other trials using growth factors, small molecules, and culture models have been developed and optimised [106–110]. In 2014, two groups succeeded in making insulin-producing cells from human embryonic and induced pluripotent stem cells in vitro [100,111]. Both groups transplanted their stem cell-derived IPCs to mice with induced insulin-dependent diabetes, in which these cells ameliorated or reversed the diabetes mellitus. Some companies are conducting clinical and pre-clinical trials in humans using human ESC- and iPSC-derived pancreatic progenitor cells, implanting them directly under the skin of the patient or encapsulated by a semi-permeable membrane [112,113]. A major concern of these techniques is that both ESCs and iPSCs can give rise to teratomas when residual undifferentiated cells are transplanted to a patient, although this is less of a concern when a semi-permeable membrane is used [114].

6.4.2. Adult Stem Cells

Adult stem cells (ASCs) are organ specific and have the ability to self-renew and to differentiate into cells of the tissue in which they are residing. The use of these cells does not raise ethical issues associated with ESCs, their culture protocols are relatively simple, and they lack tumorigenic potential, in contrast to ESCs and IPSCs [115]. Studies have shown the ability of the pancreas to increase its β-cell mass throughout life, indicating the presence of endocrine ASCs [116,117]. However, it remains unclear whether an adult pancreatic stem cell actually exists. Also, the source of the proposed ASCs remains enigmatic. They could be located either in the acinar tissue [118], ductal epithelium [119,120], or the islet itself [121,122] and could be produced by the duplication of adult β-cells [123] or via transdifferentiation of α-cells and δ-cells into β-cells [124]. Lineage tracing experiments reported the presence of Lgr5-derived (classical adult epithelial stem cells marker) cells in the mouse pancreas [125]. Culturing of these cells in vitro and the gene expression profile confirmed a ductular developmental stage, whereas further differentiation of these Lgr5-derived organoids resulted in the formation of endocrine cells. Lee et al. [126] converted human pancreatic ductal cells into a β-cell phenotype using an adenovirus-driven expression of Neurog3, MafA, Pdx1, and Pax6. Subsequently, two studies described the culture of normal and neoplastic pancreas organoids derived from tissue collected through endoscopic needle biopsies [127,128].

7. Canine Culture of β-Cells: Where Are We Now?

Although ESCs and iPSCs have been generated from canine tissue [129–134], their existence is under debate, due to lack of crucial confirming experiments. There are few publications mentioning spontaneous in vivo differentiation (teratoma) using canine iPSCs, but the crucial chimera formation has not been reported yet [94]. Regarding T1DM, the isolation and characterisation of pancreatic canine foetal cells in a 2D model cell culture system were described where the expression of the pluripotent marker NANOG, the proliferation marker PCNA, and the pancreatic transcription factor PDX1 indicated that these cells could be a source of stem cell studies [135].

Another technical procedure recently tested in dogs uses islet-like structures isolated from cultured foetal pancreases. Initially described in 1979, using rat pancreas foetal tissue [136], the cells obtained through this technique were named pseudoislets (PIs) and became an alternative source for T1DM cell therapy. Czernichow et al. (2019) investigated the potential use of canine PIs from foetal or postnatal pancreases and demonstrated their ability to produce insulin [137]. Although euglycaemia was not achieved when canine PIs were transplanted in mice, improvements in the density of β-cells produced with this technique can be useful.

A clinical trial with Neo-Islets, composed of canine pancreatic islet cells and adipose-derived multipotent stromal cells, showed their potential after transplantation in dogs with spontaneously occurring DM (Figure 3(5)) [138]. The selected dogs received 2×10^5 Neo-Islets per kilogram body weight by allotransplantation into their peritoneum and were followed-up for three years after transplantation. During this time, the blood glucose concentration and several other parameters were monitored closely. The preliminary report shows that in three out of four dogs, the blood glucose concentration and the need for exogenous insulin was significantly reduced after the infusion of canine Neo-Islets. In the fourth dog, the insulin requirement remained unchanged (12 months post-transplantation). Although glycaemic control improved, none of these dogs became insulin-independent after transplantation. Even though this technique still requires donor pancreases, the number of patients treated can be significantly increased because more than 80 therapeutic doses can be generated from one pancreas. Furthermore, no side effects were reported.

8. Discussion and Future Perspectives

In recent years, regenerative medicine has become a very promising potential solution for human T1DM. Several studies showed the ability of human ESCs and iPSCs to differentiate into insulin producing β-like cells [96,107,139,140]. This approach could also be considered in canine diabetic patients, because pancreatic organogenesis in mammals is highly similar among different species [32–34]. However, even the most effective protocols to differentiate stem cells into β-like cells to date still need improvement in cell functionality and maturation before these cells could be used to cure T1DM.

More research into the applicability of stem cells as a source for IPCs, their long-term functionality in physiological conditions, and their safe use as a therapeutic alternative is needed. New challenges are the optimisation of differentiation medium protocols to guarantee efficient production of functional stem cell-derived β-like cells, the search for a synthetic extracellular matrix, and the development of a biocompatible device that enables the release of the produced insulin while shielding the cells from the host immune response. Furthermore, efforts to produce a sufficient number of β-like cells in vitro to restore euglycaemia in vivo are essential. Also, the number of cells per dose, the best application site of the device containing IPCs, and the way to improve the biocompatibility and vascularisation of scaffolds to assure the longevity of cells within the device, while avoiding the host immune response need to be investigated in future clinical trials (Figure 4).

It is not clear if these stem cells will make their way into the veterinary clinic, given the pro and cons of various strategies summarised in Figure 3 and the remaining issues depicted in Figure 4. Most close to clinical implementation seems to be continuous blood glucose monitoring combined with insulin infusion systems. An important benefit is the practical application and the enormous gain in the quality of life. The main advantage of iPS- or mesenchymal-derived stem cell-based therapies is the ease to acquire donor cells, in contrast to the (perceived) simplicity of reprogramming. Cell-based therapies in general will face the culprit of T1DM, being an autoimmune disorder. Devices to dampen the autoimmune response are crucial to advance these therapies into clinical practice. Furthermore, screening for detecting early diabetes promotes the adoption of therapeutical measures and consequently a longer survival. One century after Banting and Best discovered insulin in dogs and the Nobel prize in physiology was awarded for the insulin research in 1923, diabetes still poses a major clinical burden for hundreds of

thousands of human and canine patients worldwide. Dogs have been instrumental in this research area, and it is time that dogs can finally benefit from their crucial role in the field of DM research.

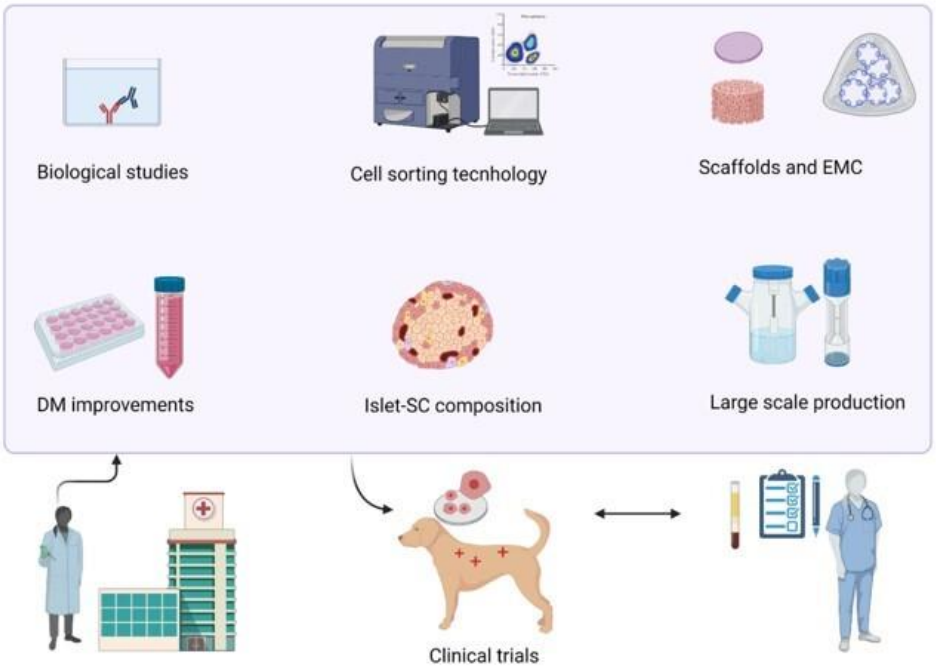


Figure 4. The remaining challenges for the use of stem cell β-like cells in type 1 diabetes mellitus therapy. In vitro studies related to the optimisation of differentiation medium (DM) and biological assays to evaluate cellular functionality are the initial steps. Cell sorting techniques used to define islet stem cell composition could be useful for future approaches. Bioengineering technology advances are required to (i) improve the biocompatibility and vascularisation of scaffolds to assure the longevity of cells within the device, while avoiding the host immune response; (ii) the replacement of the extracellular matrix (EMC) from animal origin with a synthetic hydrogel; (iii) ensure large-scale production of cells essential for future use. Clinical trials will be needed to determine the cell dose, the best implantation site, and the effectiveness of treatment. Figure created in Biorender.com.

Funding: This study was financed in part by the Coordenação de Aperfeiçoamento Pessoal de Nível Superior—Brasil (CAPES)—Finance Code 001.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Conflicts of Interest: The authors declare no conflicts of interest.

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