Review

Novel Strategies to Protect and Visualize Pancreatic β Cells in Diabetes

Esteban N. Gurzov,^{1,*} Pu Chun Ke,^{2,3} Ulf Ahlgren,⁴ Rita S. Garcia Ribeiro,¹ and Martin Gotthardt⁵

A common feature in the pathophysiology of different types of diabetes is the reduction of β cell mass and/or impairment of β cell function. Diagnosis and treatment of type 1 and type 2 diabetes is currently hampered by a lack of reliable techniques to restore β cell survival, to improve insulin secretion, and to quantify β cell mass in patients. Current new approaches may allow us to precisely and specifically visualize β cells *in vivo* and provide viable therapeutic strategies to preserve, recover, and regenerate β cells. In this review, we discuss recent protective approaches for β cells and the advantages and limitations of current imaging probes in the field.

Type 1 and Type 2 Diabetes (T1D and T2D) as a β Cell Pathology

T1D and T2D, although arising from different etiologies, are each associated with physical and/or functional loss of insulin-secreting β cells [1–3]. T1D is described as a heterogeneous inflammatory disease characterized by infiltration of the pancreatic islets with a number of autoimmune cells (CD4+ and CD8+ T cells, macrophages, dendritic cells, and B cells) [4]. Recent evidence suggests that progression of islet infiltrates promotes β cell dysfunction (reduced first-phase insulin response) and later β cell elimination, which ultimately results in the onset of diabetes. Interestingly, it is now clear that residual β cell mass can be found several years after diagnosis. Thus, a desired strategy for T1D treatment should suppress β cell autoimmunity, along with protection and restoration of the remaining β cell mass. Currently, there are no clinically approved interventional therapies for treating the underlying autoimmunity and boosting β cell survival in T1D. T2D, however, is characterized by insulin resistance following progressive decline in β cell function and accumulation of islet amyloid polypeptide (IAPP, or amylin) in the pancreatic islets [2,5]. The pathology is far more complex, as a recent study on newly diagnosed diabetic subjects postulates a reclassification into five distinct clusters based on the metabolic profile and risk of complications; three of the different cluster are currently considered T2D [6]. The treatment of T2D is mainly limited to metformin monotherapy as the initial strategy to improve insulin sensitivity. When the patient cannot control glycaemia by lifestyle and metformin, a pharmacology approach to increase β cell resistance and function will be required, as an alternative to chronic exogenous insulin injections.

The β cell mass constitutes a low percentage of the pancreas and varies considerable between subjects. The β cells are deeply imbedded and irregularly dispersed within the exocrine pancreas in the islets of Langerhans or pancreatic islets, thus virtually inaccessible for functional studies [7]. To complicate matters further, the islets are present in vast numbers and estimations between 3.6 and 14.8 million are suggested in the literature [8]. Although several advances have been made in the field, the precise molecular mechanisms leading to dysfunctional β cells in the different forms of diabetes are unknown. Even if we are able to detect the culprits of β cell dysfunction/death and eventually design a therapeutic strategy, it will still be missing the right diagnostic method, patient subpopulation, and time to use it. Thus, adequate noninvasive β cell imaging is necessary to combine with therapeutic approaches to proper follow-up of T1D and different subtypes of T2D. It is critical to identify and assess novel β cell restricted molecular targets for

Highlights

Pancreatic β cell failure is characteristic to both T1D and T2D.

The JAK-STAT pathway is a promising target to prevent autoimmune β cell destruction.

Several strategies are currently being tested to improve insulin production in T2D: preventing β cell death/dysfunction, enhancing β cell proliferation, ameliorating β cell dedifferentiation, and inducing transdifferentiation into β like cells.

Imaging and diagnostic tools are required to improve the efficacy of current therapeutic strategies aimed at restoring β cell function.

Nanotechnology provides solutions to current problems of sensitivity, efficacy, and accessibility in the diagnosis and treatment of diabetes.

¹Signal Transduction and Metabolism Laboratory, Université libre de Bruxelles, Brussels 1070, Belgium

²Zhongshan Hospital, Fudan University, Xuhui District, Shanghai 200032, China

³ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC 3052, Australia

⁴Umeå Centre for Molecular Medicine, Umeå University, Umeå S-90187, Sweden

⁵Department of Medical Imaging, Radboud University Medical Center, Nijmegen 6525 GA, The Netherlands

*Correspondence: egurzov@ulb.ac.be (E.N. Gurzov).

imaging. This could also eventually result in more efficient diagnostic tools, allowing physicians to tailor the treatment and intervene before the decline of β cells is irreversible. In this review, we summarize the most recent reports and remaining research gaps to protect β cells and monitor disease progression.

How to Resist and Survive the Autoimmune Attack in T1D?

Autoimmunity against pancreatic β cells is a key feature of T1D (Box 1). Multiple approaches for the induction of anti-inflammatory/regulatory mechanisms that would prevent autoimmunity in mouse models have been tested, with little or no beneficial effects in humans [9]. For example, insulin immunization, GAD65, anti-IL-1/IL-1 receptor antagonist, antithymocyte globulin, and anti-CD3/anti-CD20 antibody treatments prevented autoimmune diabetes in non-obese diabetic (NOD) mice, but did not show efficacy or were only partially effective in clinical trials [10]. Thus, there is an opportunity to repurpose approved drugs used to treat other autoimmune diseases in T1D, especially when there is evidence of overlapping mechanisms. There is good evidence of β cell activation of the Janus kinase (JAK)1/JAK2 and signal transducer and activator of transcription (STAT) 1 pathway in human T1D and in autoimmune diabetes mouse models [11–13]. JAK1/JAK2 inhibitors are in development or clinical use for inflammatory diseases [14]. Using these drugs to block the JAK-STAT pathway would prevent autoimmunity against β cells in T1D [15]. AZD1480, a JAK1/JAK2 inhibitor, decreased MHC class I upregulation and prevented the effect of proinflammatory cytokines on mouse and human β cells. The treatment blocked the direct interaction between CD8+ T cells and β cells and reduced immune cell infiltration into islets. NOD mice were protected from autoimmune diabetes with AZD1480 and disease was reversed in newly diagnosed diabetic mice after treatment with the drug [15]. Different JAK/STAT inhibitors (i.e., ruxolitinib and baricitinib) have been approved for rheumatoid arthritis treatment and are currently in Phase III clinical trials for alopecia areata. However, their efficacy and safety in T1D (young) patients need to be clinically tested. One caveat of the JAK-STAT modulation is the inactivation of PDL1, induced by several proinflammatory stimuli in cancer cells and by IFNs in human islets [16]. The PD-1-PDL1 system allows tolerance to pancreatic β cell antigens and, if disrupted, autoimmune destruction of the β cells may be accelerated in genetically predisposed individuals. Therefore, a noninvasive imaging approach (see later) would be critical to measure in real time β cell mass and inflammation and assess the beneficial or detrimental effect of the JAK-STAT inhibitors, minimizing risks (Figure 1).

Oxidative stress has been linked to β cell cytotoxicity and contributes to the development of autoimmune diabetes [17]. Protein tyrosine phosphatases (PTPs) are a large superfamily of

Box 1. Molecular Mechanisms of β Cell Dysfunction in Diabetes

T1D and T2D are characterized by progressive β cell failure. In Figure 2 we review the proposed mechanisms of β cell death in T1D and T2D. In the insulinitis lesion, invading immune cells produce proinflammatory cytokines, such as interleukin (IL)-1 β , tumor necrosis factor (TNF)- α , and type I and II interferons (IFNs). Cytokines can trigger chemokine induction and endoplasmic reticulum (ER) stress. The capacity to survive the inflammatory responses may depend on a balanced JAK-STAT activation to maintain normal β cell number and function. Immune cells (i.e., macrophages) and hyperglycemia can lead to an oxidative tissue microenvironment in the islets. This results in oxidation of tyrosine phosphatases, rendering them inactive and enhancing JAK-STAT activity and the inflammatory response. Interestingly, short chain fatty acids, acetate and butyrate, can increase the activity of immune regulatory cells (T_{regs}) and counterbalance the inflammation in the NOD mouse model.

Genome-wide association studies suggest that the one of the main inherited factors in T2D is reduction of β cell development and function. The mechanisms of β cell decline during the transition from obesity to T2D are unclear, with convincing hypothesis pointing to the role of IAPP aggregation, high levels of glucose and lipid toxicity, β cell ER stress due to enhanced insulin production, increased resident macrophages, and tissue inflammation-mediated destruction of β cells, among other plausible causes.

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Figure 1. Therapeutic Strategies and Corresponding Diagnostic Tool in Type 1 Diabetes (T1D) and Type 2 Diabetes (T2D). Different approaches to ameliorate the autoimmune attack and prevent β cell destruction in T1D and increase β cell mass and function in T2D. To minimize the risk of the therapy and follow the outcome, specific diagnostic tool should be put in place. Abbreviations: DYRK1A, Dual-specificity tyrosine phosphorylation-regulated kinase 1A; GLP-1R, Glucagon-like peptide-1 receptor; IAPP, islet amyloid polypeptide; JAK, Janus kinase; STAT, signal transducer and activator of transcription; TGF- β SF, transforming growth factor- β superfamily.

enzymes that dephosphorylate tyrosine phosphorylated proteins, including JAK-STAT family members. Genome-wide association and functional studies demonstrated that PTPs play an important role in the development of both forms of diabetes [18]. The low thiol pK_a of the Cys residue in the catalytic site of PTPs renders these proteins highly susceptible to inactivation by oxidative stress [18,19]. It was recently established that pancreatic oxidation of the phosphatases PTPN2 (a T1D candidate gene) and PTPN6 occurs *in vivo* due to insulinitis leading to cytokine-induced dysfunctional signaling [11,20]. Thus, it would be desirable to prevent oxidative stress and PTP oxidation to restore the anti-inflammatory potential of β cells. Importantly, the drug verapamil, targeting thioredoxin-interacting protein (TXNIP), a cellular redox regulator, improves β cell function and partially restores glucose homeostasis in a small cohort of adult subjects with recent-onset T1D [21]. The caveat with oxidative stress-based therapies is their lack of specificity and potential of unwanted side effects. Hence, the development of a combination of oxidative stress- and β cell-biomarker strategy will allow patient stratification and improve therapy outcome.

In recent years, molecular mechanisms whereby the gut microbiota influences immune responses have come to light and some authors have hypothesized that gut dysbiosis can underlie the pathogenesis of T1D [22]. A novel 'HAMSA/B' diet, based on high-amylose maize starch that has been acetylated and butyrylated to release the short chain fatty acids after microbiota fermentation, provided complete protection from diabetes in the NOD mouse model [23]. Acetate significantly decreased the frequency of autoreactive T cells in lymphoid tissues in HAMSA/B-fed NOD mice, through effects on B cells and their ability to expand populations of autoreactive T cells. Butyrate, however, enhanced the number and function of regulatory T cells and, together, acetate and butyrate boosted gut integrity and decreased serum concentration of diabetogenic cytokines, such as IL-21 [23]. Interestingly, acetate and butyrate efficiently decrease oxidative stress, as exemplified by our observation of reduction of PTP oxidation in the pancreas of

HAMSA/B-fed NOD mice (Gurzov, E.N. & Mariño, E., unpublished data). Importantly, the HAMSA/B diet has been recently tested in a cohort of subjects with established T1D and was well tolerated in the ACTRN12618001391268 ToGeTher (Type One Diabetes and Gut Therapy) trial. Thus, chemical modified foods or metabolites might represent an effective and physiological approach for countering the immunological defects and improve β cell function.

T1D results from failure of tolerance mechanisms (i.e., the elimination or silence of a diverse repertoire of specific T and B cells recognizing self-components). Exposure to antigens early in life, antigen-induced therapy, is an attractive strategy to re-establish tolerance and has shown efficacy in diabetic mouse models [24]. However, prevention studies in humans lack robust evidence due to the difficulty of selecting the predisposed subjects and evaluating autoimmune attack to the β cells in the pancreas. In addition, preclinical studies have suggested that combined administration of antigenic therapies (e.g., islet autoantigens), immune modulators (e.g., anti-CD3 antibody), and restoration of β cell mass may be required for efficacy in patients with already established pathology. To date, there is no therapeutic approach in the clinics that can increase β cell mass and function in T1D.

How to Protect β Cells and Improve Insulin Secretion in T2D?

There are four main strategies aiming to restore insulin secretion in β cells in T2D: preventing β cell death/dysfunction, enhancing β cell proliferation, ameliorating β cell dedifferentiation, and inducing transdifferentiation into insulin-producing β -like cells (Box 1, Figure 2).

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Figure 2. Mechanisms of β Cell Dysfunction and Death in Type 1 Diabetes (T1D) and Type 2 Diabetes (T2D). The autoimmune attack is responsible for β cell destruction in T1D. The disease is heterogeneous in individuals with variable amount of insulinitis, progression, and immune cells involved. It is unclear which precise event triggers autoimmunity. In T2D, several causes have been proposed as the 'culprit' of β cell dysfunction and death. It is probably a combination of several different factors contributing to disease development. Abbreviations: APC, Antigen-presenting cell; ER, endoplasmic reticulum; IAPP, islet amyloid polypeptide; IFN, interferon; JAK, Janus kinase; PP, pancreatic polypeptide; ROS, reactive oxygen species; STAT, signal transducer and activator of transcription.

Similar to what is known for β -amyloid or α -synuclein, IAPP oligomers are the most toxic species among the aggregation states of the peptide and β cells are especially prone to cell membrane damage and generation of reactive oxygen species by IAPP oligomers (Box 2, Figure 3), contributing to loss of β cell function and mass in T2D [25]. There have been a number of strategies developed to curb the amyloid aggregation and hence β cell toxicity elicited by IAPP, with small molecules such as epigallocatechin-3-gallate, resveratrol, 2,8-bis-(2,4-dihydroxy-phenyl)-7-hydroxy-phenoxazin-3-one [26,27] or, more recently, with nanomaterials such as OH-terminated dendritic polymers, star polymers, gold nanoparticles (AuNPs), silica nanohelices, or graphene quantum dots [28]. The working principle of these approaches is to out-compete the association between IAPP monomers with stronger binding between IAPP and an inhibitor, mediated by H-bonding, hydrophobic, or electrostatic interactions. In one case, fresh monomeric IAPP injected to zebrafish embryos was sequestered by carbon nanotubes coated with the amyloid fragments of whey protein β -lactoglobulin via double protein coronae, thereby reviving hatching and development of the organism from cell degeneration [29]. In a more recent study, protein dysregulation in β cells exposed to IAPP was mapped by liquid chromatography-tandem mass spectrometry, which was then mitigated by incubating the cells with graphene quantum dots [30].

In the past 20 years, significant efforts have been directed toward identifying mechanisms that can trigger β cell proliferation for therapeutic purposes in diabetes. Such efforts have highlighted the difficulty of starting replication in differentiated adult β cells. Recently, the laboratory of Andrew Stewart successfully combined two different strategies, namely the inhibition of the enzyme dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A), which stimulates cell-cycle activators, and the inhibition of the transforming growth factor- β superfamily (TGF- β SF), which represses cell-cycle inhibitors. The combination induced a synergistic increase in replication of mature human β cells [31]. They obtained unprecedented β cell proliferation rates in the 5–8% range. Moreover, the same group was able to improve the efficacy therapy of Glucagon-like peptide-1 receptor (GLP-1R) agonists when combined with DYRK1A inhibitors, synergistically increasing proliferation and function of β cells derived from T2D individuals [32]. These findings could make a treatment to restore β cell deficiency in diabetes and merit further investigation. Another active field of preclinical research for β cell proliferation

Box 2. Biology and Nanomaterials for IAPP Mitigation in Diabetes

The β cells are a unique environment, possessing a pH of ~ 5.5 and maintained by metabolic factors and ligands common to all mammalian cells. In the intracellular space, insulin and IAPP are synthesized at a molar ratio of $\sim 100:1$. Insulin is stored primarily as zinc insulin hexamers in the central core of β cell granules, while IAPP is a highly amyloidogenic 37-residue peptide, with a disulfide bond connecting residues 2 and 7 (Figure 3B). Specifically, the residues 20–29 of IAPP are the primary amyloidogenic segment, while residues 8–17 are the secondary amyloidogenic segment of the peptide [68] (Figure 3B). The acidic environment of β cells ensures the positive charge of His18 of IAPP to inhibit its amyloid aggregation, which is further stabilized by insulin, Zn^{2+} [69] and C-peptide at specific stoichiometric ratios to IAPP and Zn^{2+} [70]. Upon release from β cells, however, IAPP experiences a sudden rise in environmental pH, which triggers a structural transition of the peptide from its monomeric state to oligomeric, protofibrillar, and amyloid fibrillar forms, further prompted by cell membranes in contact with the peptide N terminus. A range of inorganic and organic nanomaterials have been utilized for mitigating the amyloidosis of amyloid proteins in protection of neuronal and β cells from degeneration [28]. The common characteristics of an effective nanostructured inhibitor include their good biocompatibility, long bio-circulation, strong ability in initiating H-bonding and/or hydrophobic interactions with amyloid proteins, and a potential against reactive oxygen species. Among the inorganic nanomaterials tested against β cell degeneration, AuNPs, silica, and iron oxide appear promising also due to their use in a range of biosensing, bioimaging, and drug delivery applications. For organic nanomaterials, graphene quantum dots, carbon nanotubes, and polymeric nanoparticles have shown great efficacies in sequestering toxic IAPP oligomers and protofibrils *in vitro*, *ex vivo*, and *in vivo* (Table 1). The future of this emerging field lies in the design and optimization of functional nanocomposites for performing multiplexing theragnostic functions *in vivo*, from IAPP amyloidosis inhibition to sensing of insulin, glucose, and β cell mass against diabetes.

Figure 3. Biophysical Principle of Islet Amyloid Polypeptide (IAPP) Amyloid Aggregation (Amyloidosis), Their Implications and Mitigation with Tailor-Designed Biocompatible Nanoparticles. Central to amyloidosis inhibition is the suppression of peptide self-assembly (A and B), triggered by adverse cellular conditions (A, left side), with elevated peptide-nanoparticle interaction (B). Abbreviations: ROS, Reactive oxygen species; T2D, Type 2 diabetes.

is the use of microRNA to increase cell replication and improve function [33,34]. The challenge in β cell proliferation is to determine whether the strategies will work in β cells from different subjects and if the expansion can be selectively targeted to β cells and not to other cells inducing neoplasia. Interestingly, increased β cell proliferation may also prevent the development of T1D [35].

Alterations in the identity of the β cells within the pancreatic islet may also contribute to T2D [2,36]. Chromatin and genetic models for β cell dedifferentiation in T2D have been recently proposed [37–39], but the therapeutic significance of the findings still remains distant. Restoration of adiponin, an adipokine, in diabetic mice preserves β cell mass by enhancing survival and maintaining transcriptional identity of the β cells [40]. The mechanism is mediated by decreased activity of DUSP26, a critical phosphatase that is upregulated in β cells in diabetes and affects cell survival. Unfortunately, there are no specific DUSP26 inhibitors available for clinical trials. An alternative to prevent β cell dedifferentiation is to increase transdifferentiation from pancreatic-progenitors [41], acinar [42], or endocrine [43] cells into insulin-secreting β -like cells. However, without clear-cut cell-lineage tracing markers, identification of the transition between different cells in the human pancreas is difficult to interpret. Another limitation of the current studies is drawing conclusions from autopsy and/or organ donor islets by histology or fluorescence-activated cell sorting analysis. The use of novel preclinical 3D imaging, pancreas slides, and metabolic profiling (Box 3), would be desirable to provide accurate β cell quantification and islet cell identity in pharmacology/genetic approaches.

Molecular Imaging with β Cell-Specific Probes: The Quest to 'Light up' β Cells in the Pancreas

The novel existing and future therapies may change the way we treat diabetes. Towards this goal, firstly we need to better understand the relationship between β cell mass and function, and secondly, we need to employ appropriate imaging technologies for elucidating the pathophysiology of the disease while monitoring therapies concurrently (Figure 1).

Noninvasive molecular imaging of β cells in the pancreas requires a stable and well-expressed protein that can be targeted by a suitable radiotracer. This protein should not be expressed in other endocrine or exocrine cells. Nuclear imaging techniques [e.g., positron-emission tomography (PET), especially for clinical imaging, and single-photon computed tomography (SPECT)] are suitable techniques for β cell imaging, as they entail high sensitivity (in the pico/nanomolar range), (sub)millimeter spatial resolution (especially SPECT imaging in small animals has a resolution of about 300 micrometers), and proven performances in preclinical models. For an overview about the imaging techniques, we refer to the following reviews [44,45].

An increasing number of tracers/compounds for β cell-specific labeling that hold promise for clinical use are being explored [45,46]. Regardless, if such compounds are intended for use as imaging agents and/or for clinical interventions, their uptake specificity and effect must be carefully evaluated, as must the reliability of the read-out from the imaging platforms for which they are intended. An impediment for most noninvasive imaging approaches is, relatively speaking, the low resolution of these techniques to resolve individual islets. For assessments of overall β cell mass, this does not have to impose a problem and it may even be favorable in terms of data handling if the radiotracer is specifically accumulating in β cells as the total signal in the pancreas can then be taken as a

Box 3. Preclinical Approaches for β Cell Evaluation in Diabetes

Given the relatively incomplete picture of the natural and pathologic islet distribution of the human pancreas, it is advantageous if regional differences or even aspects of islet composition and function on the level of individual islets could be analyzed in preclinical studies. A technique that has already demonstrated utility in the evaluation of tracer read-out is optical projection tomography (OPT) [55,71]. This technique may be seen as the optical equivalent of X-ray computed tomography, which allows for the generation of quantitative 3D assessments of essentially any protein expression pattern that could be detected by antibodies in optically cleared specimens (Figure 1). Owing to the possibility of calculating absolute islet numbers and even their individual volumes in pancreatic specimens labeled with islet-specific markers (see e.g., [72,73]), this technique may be considered as a gold standard against other imaging assessments. Indeed, we have shown that ^{111}In -exendin-3 SPECT imaging and OPT linearly correlates and both techniques are more reliable than histological assessment of β cell mass [74]. A recent standardized approach has been postulated to define criteria that β cell traces should fulfill [45].

Another important aspect of evaluating imaging agents/tracers is their potential impact on islet longevity and function. New techniques for *in vivo* optical and spectroscopic assessments of islet function may become useful tools for such undertakings. For example, islets grafted to the anterior chamber of the eye become vascularized and provide a 'mirror image' of their endogenous counterparts. This has included xenografting both of human islets into rodents and grafting in higher primates. By this technique, several aspects of islet biology have become possible to study in individual islets *in vivo* and over time by confocal microscopy [75]. A more recent application of this transplantation technique has utilized microRaman spectroscopy to assess biochemical changes in the grafted islets [76]. By multivariate analyses of the Raman data, details of the islets' biochemical composition and function may be determined, which could potentially provide information on aspects of the islets' response to a therapeutic agent. It is important to consider that the choice of the animal model will influence the outcome.

The development of pancreatic tissue slices has been shown to be useful to study human islet function under near-physiological conditions [77]. The authors showed loss of insulin secretion and β cell mass at different stages of the disease in pancreas from organ donors. Importantly, one sample of a recent-onset T1D donor has reduced insulin secretion due to β cell dysfunction but not to reduced β cell mass, suggesting that β cells may be preserved at disease onset in some patients and their function can be eventually restored.

Jointly, the earlier listed techniques may thus become valuable complements for evaluating noninvasive techniques for the quantitative, functional, and therapeutic assessments of the β cells.

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Figure 1. Optical Projection Tomography (OPT) Image of Pancreas from Non-Obese Diabetic (NOD) Mice.

Optical mesoscopic imaging enables detailed assessments of the islet distribution and volume. The image shows a 3D reconstruction of a mouse pancreas (splenic lobe) from a type 1 diabetes model (NOD mice) generated by OPT. The signal from insulin-specific antibodies (in red) and aggregates of infiltrating CD3 positive cells (in green) are shown. The outline of the pancreatic parenchyma is generated based on its autofluorescence and the rendering is made transparent by imaging software. Due to the tomographic nature of the technique, each islet's volume, shape, and 3D coordinates may be determined throughout the volume of the investigated specimen. As such it can serve as a gold standard when evaluating quantitative read-outs by other imaging modalities. In theory, any protein expression pattern could be analyzed also using specific antibodies against 'functional' markers.

measure of β cell mass. Among the tested imaging biomarkers, GLP-1R is a promising target molecule, as it is reported to be expressed in human pancreatic β cells at high densities [47]. It has been shown that radiolabeled exendin is a promising tracer to visualize β cells in rodents and humans [48,49]. Importantly, inflammatory conditions (i.e., insulinitis) do not influence the uptake in the β cells, which has always been a major concern for the use of the tracer as a diagnostic tool in T1D [49,50]. Moreover, there is no relevant uptake of the tracer in the exocrine pancreas and no contribution of α cell mass to the uptake. It therefore appears that radiolabeled exendin is the most specific tracer currently available. GLP-1R binds specifically with GLP-1 secreted from intestinal L-cells by the stimulation of nutrients, leading to the promotion of glucose-dependent insulin secretion. GLP-1R agonists are now preclinically and clinically tested in the treatment of T1D and T2D [51]. Hence, the GLP-1/GLP-1R axis is both a therapeutic and imaging/diagnostic target in diabetes research.

The G protein-coupled receptor 44 (GPR44) was recently identified in the pancreas as β cell specific [52]. GPR44 is accessible by potential imaging agents as it is located in the outer cell membrane [53]. GPR44 PET tracer [^{11}C]AZ12204657 has more than ten times higher receptor-specific binding to human islets compared with exocrine preparations [54]. However, the average density of available GPR44 in the pancreas is low, due to the low proportion of islets in the tissue, and individual islets were not identified by PET. A recent study with radiolabeled zinc transporter 8 (ZnT8)-targeting antibody fragment using ^{125}I , reported that the binding affinity of this probe to insulinomas and the pancreatic accumulation of this tracer was higher than ^{125}I -labeled exendin-4 [55]. These initial results indicated that antibody fragments may be promising alternatives for β cell imaging. Interestingly, engineered antibody fragments have also been developed for immune cells [56]. This would eventually allow targeting of autoimmune cells combined with β cells/islets for diagnostics in T1D. The caveat is the 'dilution' of autoreactive cells in the pool of immune cells and the lack of available markers to identify them.

Nanobodies (15 kDa), consisting of the variable domain of camelid heavy chain antibodies, have advantageous features such as small size, high solubility and stability, tissue penetration *in vivo*, and fast blood clearance. A novel β cell biomarker expressed on the cell surface of pancreatic endocrine cells, named dipeptidyl peptidase 6 (DPP6), was identified and targeted with nanobodies [57]. DPP6 is expressed at the plasma membranes of β and α cells, thereby allowing their detection by SPECT/CT. Its expression is not influenced by exposure to proinflammatory cytokines or metabolic stress, features of T1D [58]. A novel generation of synthetic nanobodies, namely sybodies, are designed to mimic the diversity of camelid nanobodies. Sybodies are inexpensive and can be entirely produced *in vitro* in a short 3-week time frame [59], but their use for imaging needs to be validated. Thus, nanobodies and nanobody-based molecules will open new opportunities in imaging and diagnostic research in the near future.

Nanoparticles and Novel Emerging Technologies Targeting β Cells and Insulin Delivery

The use of biocompatible nanomaterials has recently become an emerging strategy as diagnostic and therapeutic tools in a range of metabolic diseases. The development of nanoparticles targeting specifically β cells (Table 1) may overcome some of the sensitivity limitations of nuclear probes mentioned earlier. Ideally, such nanoparticles should target a receptor that is abundant at the surface of the β cells or, alternatively be incorporated by a specific transporter. Also, the biodistribution and binding of the target molecule should not be affected by the chronic inflammatory and particular metabolic environment in T1D or T2D.

A pH-sensitive nanoprobe has been developed for specific pancreatic β cell imaging, exploiting its unique acidic intracellular environment [60]. Therein, magnetic iron oxide nanoparticles

Table 1. Novel Nanomaterial to Target and Protect β Cell Mass in T1D and T2D

Nanomaterial	Loaded/stabilized with	Type of study	Comments	Refs
PLGA nanoparticles	Iron oxide and indocyanine green	<i>In vivo</i>	Monitoring of isolated islets by MRI and optical imaging system	[62,78]
Liposomes	Iron oxide	<i>In vivo</i>	Improved labeling of pancreatic islets and longitudinal follow-up by MRI	[79,80]
Microparticles	ATRA/TNF- α	<i>In vivo</i>	Modulation of T _{reg} differentiation, stability, and proliferation	[81]
Dendrimers (PAMAM-OH)		<i>In vitro</i>	Binding of monomeric islet amyloid polypeptide with the hydrophobic interior of the dendrimers	[82]
Star polymers (poly(2-hydroxyethyl acrylate) nanostructures)		<i>In vitro</i>	Binding of monomeric islet amyloid polypeptide with the hydrophobic branches of the star polymers	[83]
Gold nanoparticles	PEG Citrate	<i>In vitro</i>	Aggregation of islet amyloid polypeptides	[84]
Carbon nanotubes	β -Lactoglobulin	<i>In vivo</i>	Sequestration of toxic islet amyloid polypeptide aggregates via double protein coronae	[29]
Graphene quantum dots		<i>In vitro</i>	Rescue of protein dysregulation of pancreatic β cells exposed to human islet amyloid polypeptide	[30]
Silica nanoribbons	Carboxylated	<i>In vivo</i>	Sequestration of islet amyloid polypeptide via the chirality of slice nanohelices	[85]
Proteoliposomes	ZnT8 self-antigen	<i>In vitro</i>	ZnT8A detection using a proteoliposome-based full-length ZnT8 self-antigen immobilized on a plasmonic gold platform	[86]
Insulin-timer fusion protein		<i>In vivo</i>	Real-time and simultaneous imaging of β cells and T cells and monitoring of islet transplantation	[87]

(IONPs) were subjected to surface modification by coating brushed polymers described as a pH-sensitive fluorescence ‘Turn On’ probe. Initially the probe was nonfluorescent due to the IONP surface energy transfer resulting from their broad UV-vis absorption. When in β cells, the imine bond was cleaved off due to the presence of a vast number of granules, the pH of which was at 5.5 for insulin storage (Box 2). The dye was hence released, emitting strong green fluorescence when irradiated by one-photon (430 nm) or two-photon (880 nm) excitation. According to the signal-to-noise ratios, the fluorescence in pancreatic β cells became highly distinguishable from that in other types of mammalian cells within 3 h. The strategy is a proof-of-concept that radiolabeled pH-sensitive probes may be developed in the future for β cell mass quantification.

Submicron-sized nanobubbles, able to penetrate through the microvasculature of injured tissue, have been recently used for contrast-enhanced ultrasound in the pancreas [61]. The increased islet-vascular permeability during the progression of insulinitis in mouse models of autoimmune diabetes allowed lipid-based nanobubble to accumulate within the islets. The authors showed an increase in ultrasound contrast signal originating from the islets, which correlated with disease progression [61]. As ultrasound is a cost-effective and safe medical imaging modality, it may provide a convenient approach to examine human islet microvascular permeability in patients in the asymptomatic phase of T1D. The real sensitivity of the technique in humans needs to be established; however, as insulinitis in T1D is much less pronounced compared with the NOD mouse model, the technique may not be sensitive enough for diagnosis.

Multimodal imaging, rather than single-modal imaging technique, is a powerful approach for precisely evaluating and validating the efficiency of potential β cell imaging agents. A recent study monitored the fate of pancreatic islets labeled with bimodal poly(lactic-co-glycolic acid) nanoparticles after transplantation into artificial scaffolds using trimodal imaging (F-19 MRI, fluorescence, and bioluminescence imaging) [62]. Importantly, the bioluminescence reporter constructor was a

direct marker for pancreatic islet viability and was used to validate the F-19 data *in vivo*. Together, the BLI and F-19 MRI data could address the main issue about clearance of probes from dead islets/cells and their contribution to false positives in the MRI data. Another recent study investigated the physical interactions between IAPP and AuNPs grafted with citrate and poly(ethylene glycol) of different molecular weight (PEG400 and PEG3000) [63]. They found a distinct anticorrelation between increased IAPP aggregation and decreased spectral red shifts incurred for the surface plasmon resonance (SPR) of the AuNPs. Moreover, Jurkat cells exposed to IAPP and AuNPs were characterized by quantifying their cytokine secretions with a localized SPR immunoassay, where a peak response was registered for the most toxic IAPP oligomers and was most suppressed by citrate-coated AuNPs. This study demonstrated the potential of using functionalized nanoparticles as a platform for the facile examination of protein aggregation and their induced immune response associated with amyloid diseases.

Improved insulin delivery can enhance β cell performance and has been a major effort in the management of diabetes [64]. To circumvent the issues of poor patient compliance and lack of real-time glucose control associated with daily insulin injections, 'closed-loop' insulin delivery has been demonstrated with nanotechnologies in recent years, primarily based on glucose sensing and the responsive release of insulin. Representative developments in this area include: (i) an injectable polymeric network assembled from oppositely charged dextran nanoparticles, which released their insulin load upon the catalytic generation of gluconic acid under hyperglycemic conditions in mice [65]; (ii) 'smart' microneedle-array patch containing glucose-sensitive vesicles, which altered their hydrophobicity through bio-reduction under hypoxic conditions to disassemble and subsequently deliver insulin [66]; and (iii) polymeric core-shell nanogels, which collapsed to unload insulin upon the triggers of glucose or H_2O_2 [67]. Improved antifouling and efficacy as well as reduced invasiveness remain challenges in the clinical translation of these exciting nanotechnologies.

Concluding Remarks and Future Perspectives

There has been a strong push in recent years to develop novel strategies to protect β cells, to increase β cell mass/function, and to design new visualization techniques for β cell imaging and diabetes diagnostics. Unfortunately, most of those efforts have been made in individual laboratories without a collective synergy. We strongly believe that comprehensive research projects aiming to develop multiplexing and streamlined protective and corresponding visualization/diagnostic strategies are urgently required for therapeutics in both T1D and T2D. Hence, funding agencies and foundations should prioritize long-term projects, which support close multidisciplinary collaboration between scientists of different expertise to overcome the pathology (see Outstanding Questions).

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Outstanding Questions

Is it safe to target JAK-STAT, proliferation, and developmental cell pathways in β cells without side effects?

Can nanoparticle inhibitors be designed with high potency, satisfactory delivery efficacy, long bioavailability, excellent biocompatibility, and biodegradability?

How do we design molecular imaging probes with high targeting capacity, high quantum efficiency, and low toxicity?

Can we synergistically develop therapeutic and diagnostic strategies in diabetes?

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