

Noninvasive Imaging Techniques in Islet Transplantation

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Abstract Since the Edmonton trials, insulin independence can reproducibly be achieved after islet transplantation. However, a majority of patients resume insulin treatment in the first 5 years after transplantation. Several mechanisms have been proposed but are difficult to pinpoint in one particular patient. Current tools for the metabolic monitoring of islet grafts indicate islet dysfunction when it is too late to take action. Noninvasive imaging of transplanted

islets could be used to study β -cell mass and β -cell function just after infusion, during vascularization or autoimmune and alloimmune attacks. This review will focus on the most recent advances in various imaging techniques (bioluminescence imaging, fluorescence optical imaging, MRI, and positron emission tomography). Emphasis will be placed on pertinent approaches for translation to human practice.

Keywords Islet transplantation · Imaging · Bioluminescence Imaging · Magnetic resonance imaging (MRI) · Positron emission tomography (PET) · Superparamagnetic iron oxide (SPIO) nanoparticles · Fluorescence optic imaging · Islet transplantation monitoring

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Introduction

Within the past decade, islet transplantation has become one of the most effective approaches, after pancreas transplantation, to treat type 1 diabetes. This is largely the results of the impact of the landmark “Edmonton protocol” that allowed consistent achievement of insulin independence, mainly by transplanting a high islet mass to offset the poor engraftment rate [1]. Low islet engraftment is thought to be the result of islet damage caused by the isolation procedure, ischemia-reperfusion injury, and nonspecific inflammatory processes such as an instant blood-mediated inflammatory reaction [2].

The excellent results of the Edmonton protocol at 1 year have been dampened by the 5-year outcomes reporting insulin independence persistence in only 10% to 15% of patients, although graft function was retained (positive C-

peptide) in most of the recipients [3]. Proposed mechanisms for long-term islet graft loss are allogeneic immune response, recurrence of autoimmunity, immunosuppressive drug toxicity, lack of β -cell regeneration, or exhaustion of the islet graft [4–8].

Compared with other organ transplants, the mechanisms of islet engraftment and islet loss are poorly understood. This is a result of the lack of monitoring tools able to allow early detection of graft damage. Classically used metabolic parameters (glycemia, basal and stimulated C-peptide levels, or arginine-stimulated insulin levels) are late markers of islet graft dysfunction and do not provide an idea of the islet loss mechanisms [9]. Current routine immune monitoring is based on the assessment of alloimmune [10] and autoimmune antibodies [11] reflecting only humoral immunity. Moreover, whereas the importance of anti-HLA antibodies is emerging as a factor of islet graft loss [12], the significance of autoantibodies is unclear, as their correlation with clinical outcomes is not well documented [6]. Cellular immunity monitoring appears to be of greater interest, but cell-based assays are more difficult to set up and are currently under experimental investigation [13]. After intraportal infusion, liver biopsy shows islet tissue in only 31% of cases without relevant mononuclear cell infiltration, even in the case of ongoing islet loss [14].

Similar considerations apply to preclinical type 1 diabetes. Although remarkable advances have been made in the recent years in understanding the pathogenesis of type 1 diabetes, clinical tools to monitor islet mass in humans are still lacking.

In this context, direct visualization of native and transplanted islets has broadened the scope of diabetes research. Because of small islet size (50–300 μm) and large distribution area in the pancreas or the liver, noninvasive islet imaging is very challenging. In islet transplantation, the ideal imaging technique should have high resolution to be able to detect single islets in the liver, giving an islet- or β -cell-specific signal that would be easily and accurately quantified, and of course, correlated to islet functionality and islet transplantation outcome. Moreover, to allow graft rescue in the case of allograft rejection, the decrease of signal must be observed earlier than the alteration of the usual graft function parameters. Finally, to be translated to human clinical practice, safety must be demonstrated both for the recipients and the islet graft. Since the last review on this topic in *Current Diabetes Reports* published in 2007 [15], some progress has been made thanks to modern diagnostic equipment, providing an ever-growing sensitivity. This review will summarize the major recent results in the development of noninvasive in vivo islet imaging techniques in the field of islet transplantation (Table 1).

Optical Imaging

In vivo optical imaging uses light as a source of contrast. In the field of islet transplantation, the use of bioluminescence and fluorescence optical imaging has been reported for the study of islet transplants in mice. However, due to low penetration depth of the light signal, this method is not about to be applicable to human studies.

Bioluminescence Imaging

Bioluminescent cells are modified to express the luciferase enzyme gene, either after in vitro islet transfection with a viral vector [16], or by generating transgenic mouse strains expressing the luciferase gene under the regulation of the insulin promoter (FVB-RIT-Luc and FVB-MIT-Luc mice) [17, 18]. For imaging, the luciferase substrate, injected just before image acquisition, is oxidized by luciferase in luciferase-expressing cells, in an oxygen- and ATP-dependent manner. The product of the reaction emits light that can be detected and quantified in vivo. However, as light attenuates very strongly when it propagates through the tissue before reaching the detector (0.5 cm), bioluminescence imaging (BLI) can only be used in mice.

The Vanderbilt University group has recently shown that the BLI signal from MIT-Luc mice was very well correlated with the number of syngeneic islets in vitro and in vivo after transplantation under the kidney capsule. However, the correlation was lower when islets were transplanted in the liver, probably because of higher light attenuation, sparse islet distribution throughout the liver, and perhaps higher islet loss after intraportal infusion [19]. Chen et al. [17] reported that after allogeneic islet transplantation in streptozotocin (STZ)-induced diabetic mice, BLI intensity began to show a progressive decrease approximately 5 days before the rise of hyperglycemia. One day before the diagnosis of rejection based on hyperglycemia, signal was decreased by 67%. Anti-lymphocyte serum (ALS), injected when a decrease in BLI signal of more than 30% was observed, allowed the islet graft to be saved in 60% of cases, compared with 0% when ALS was given after a 20% glycemic rise. In the failed transplants that were not salvaged by ALS therapy, rejection occurred 53.5 days after transplantation, compared with 22.1 days when rejection diagnosis was based on glycemia.

Despite these interesting results, some issues remain to be addressed. In the transgenic mouse models, bioluminescence measurements can be influenced by extracellular glucose: light emission from isolated islets cultured overnight in high glucose concentrations was greater in vitro, but neither isolated islets nor animals acutely exposed to high glucose (intraperitoneal glucose tolerance test) have increased BLI emission [19]. However, following STZ

Table 1 Summary of transplanted islet imaging techniques

Imaging technique	Application domain	Advantages	Disadvantages
BLI	Research tool in mice	Very sensitive Correlated to the functional mass	Low spatial resolution Possible interaction with glycemia Less accurate in the intraportal model
MRI		No radiation Good spatial resolution	Low sensitivity
SPIO-labeled islets	All animal models Described in humans	Feasibility in humans Quantification Correlated to the islet mass	Pretransplantation labeling Long-term labeling persistence in intraportal transplantation unclear Correlation with islet function less demonstrated
Gadolinium-labeled islets	Described in mice Could be used in humans	Feasibility in the intraportal model	Pretransplant labeling Nonpersistence of the labeling agent after 60 days
Encapsulated islets	Described in mice and swine No use in humans	Better sensitivity No or less immunosuppressive drugs	No correlation with the islet function
Manganese enhanced	Described in mice Used in humans for liver disease	No pretransplant labeling Correlated to the islet functional mass	Use for native pancreas imaging Not accurate for intraportal transplantation
Islet vascularization imaging	Described in mice Difficult to translate in humans	Potentially a good surrogate marker for early graft function	Requires high-field MRI Difficult to quantify in the intraportal model
PET imaging		High sensitivity	Islet and recipient irradiation Low spatial resolution
[¹⁸ F] FDG	Described in rats and humans	Imaging just after islet infusion	Pretransplant labeling Short half-life
Noninvasive reporter probe imaging	Described in mice Not directly applicable to humans	Imaging of islet functionality	Pretransplant islet transfection Time limited technique Gut uptake
GLP-1 receptor PET imaging	Used in animal models Used in humans	Imaging of endocrine pancreas Potentially applicable to intraportal liver transplant	Islet specificity and sensibility in intraportal transplantation unclear

BLI bioluminescence imaging; [¹⁸F]FDG ¹⁸F-fluorodeoxyglucose; GLP-1 glucagon-like peptide-1; PET positron emission tomography; SPIO superparamagnetic iron oxide

treatment, the decline in BLI was less than the decrease in β -cell mass assessed by histology (78% vs 96%). In these models, luciferase expression is under control of the insulin promoter fragment and can be influenced by the glucose level, particularly in the setting of chronic hyperglycemia. Another issue is that despite the possibility of three-dimensional reconstruction to precisely define the location of the bioluminescence source [19], the resolution of the signal is low and does not allow detection of single islets scattered throughout the liver. BLI can be a useful research tool in islet transplantation in the mouse model and is more accurate for subcapsular islet grafts. The influence of glycemia needs to be clarified in the MIT and RIT transgenic mice.

Fluorescence Imaging

Evgenov et al. [20] described another application of optical imaging to human islet transplantation in mice using a near infrared fluorescent dye combined with dextran-coated superparamagnetic nanoparticles. Islets were labeled by overnight incubation and grafted under the kidney capsule of immunodeficient mice. The signal could be detected in vivo and confirmed by histologic analysis as the fluorescence remained visible on confocal fluorescence microscopy after tissue fixation. Additionally, contrary to BLI, no substrate injection was required before imaging.

Fluorescence imaging has also been used in vivo to visualize islets transplanted into the anterior chamber of the

eye by using the cornea as a natural body window. Speier et al. [21] transplanted islets from transgenic mice expressing enhanced green fluorescent protein under control of the rat insulin-1 promoter (RIP-GFP). By using intraocular and intravenous injections of fluorescent dyes, they were able to study islet engraftment and revascularization, β -cell functionality (calcium handling), and β -cell death. Recently, Perez et al. [22] transplanted unlabeled allogeneic islets in the anterior chamber of a baboon. Islets were implanted in the iris and could be observed using slit-lamp examination. Fluorescein angiography of the anterior chamber revealed islet revascularization. In this preclinical study, no severe ocular adverse effects were reported, but only 20,000 islet equivalents were transplanted.

Magnetic Resonance Imaging

In the field of islet transplantation, MRI is of growing interest. Its high spatial resolution and penetration depth, and lack of ionizing radiation make studies directly translational to humans. But because MRI cannot naturally distinguish the transplanted islets from the surrounding liver tissue, the addition of a contrast agent is required.

Superparamagnetic Iron Oxide Nanoparticles

In 2004, Jirak et al. [23] were the first to show that transplanted islets could be detected in the liver of rat with a dedicated rodent 4.7T MR system. Islets were labeled *in vitro* before transplantation using superparamagnetic iron oxide (SPIO) nanoparticles (ferucarbotran; Resovist®, Schering AG, Berlin, Germany) at a concentration of 137.5 $\mu\text{g Fe/mL}$. SPIO nanoparticles are commercially available contrast agents used for the detection of liver lesions. Their shortening effect on the T2 relaxation time of surrounding protons gives a strong hypointense signal on T2-weighted images (Fig. 1). Ferucarbotran and ferumoxide (Endorem®, Guerbet, Villepinte, France; or Feridex®, AMAG Pharmaceuticals, Lexington, MA) are the two clinical-grade carboxydextran- or dextran-coated SPIO nanoparticles tested as islet transplant labeling agents.

SPIO Labeling Studies

After *in vitro* incubation with islets, SPIO nanoparticles can be detected by electronic microscopy in the lysosomes of β cells, but also in other islet cells (α cells, δ cells, and islet macrophages), and also in the interstitial spaces around the islet cells [24–27]. The accumulation of SPIO nanoparticles in islets seems to depend on the iron and cell concentration in the culture medium and the incubation time [23]. As shown by Evgenov et al. [26], iron distribution within islet

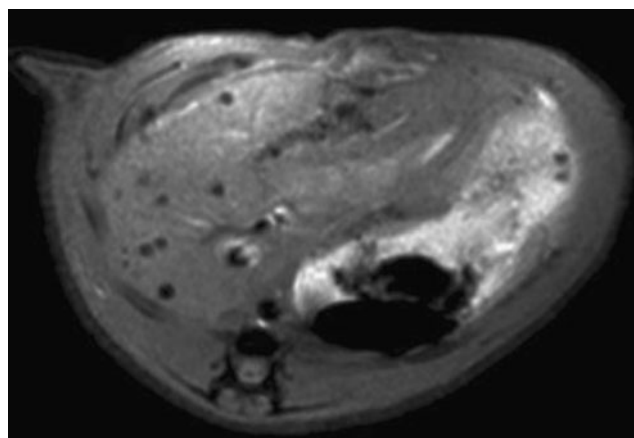


Fig. 1 3T MRI of ferucarbotran-labeled islet transplanted in the liver of a rat (T2*-weighted gradient echo sequences) (Berney, Personal data)

cells is not uniform, ranging from 10% to 70%. Recent studies tried to improve efficacy of islet labeling by using liposomes or polyethyleneimine [28, 29]. It was shown that isolated human and rodent islets can be labeled with SPIO nanoparticles without impairing their insulin-secreting capacity or viability [24, 25, 27].

Evgenov et al. [30] showed that the iron load, characterized by a short T2 relaxation time, was higher in pure islets compared with exocrine tissue alone. However, labeling exocrine tissue reduced its T2 relaxation time, suggesting that exocrine tissue can also be labeled by SPIO nanoparticles. However, even if exocrine tissue was slightly labeled, no signal difference coming from the islet graft was observed 2 days after intraportal transplantations of a same islet number of different purity (98% and 50%) in immunocompromised nonobese diabetic (NOD)-scid mice. Higher rates of apoptosis and graft macrophage infiltration in the 50% purity transplants argued for a rapid clearance of exocrine cells without MRI signal difference after day 2. However, in that article, when low (20%) and high purity preparations were incubated with the same number of islets, the iron-to-protein ratio was lower in the low purity preparation, probably reflecting higher cell concentration in the culture medium with a lower islet iron uptake.

Signal Quantification

In earlier studies, the quantification of SPIO-labeled islets in rodent liver was made manually by counting the number of hypointense spots in 8 to 13 slices, with a slice thickness varying from 0.5 to 2 mm [27, 30]. This quantification method showed an excellent correlation with the number of transplanted islet equivalents in the liver of NOD-scid mice (from 200 to 1,000), with approximately three or four islets clusters visualized for 10 transplanted. However, in a clinical application perspective, manual counting is very

difficult in human examinations because of higher volume of the liver with higher number of islets. In a pilot study in humans, Saudek et al. [31•] used manual counting on 72 slices covering the whole liver. Recently, Medarova et al. [32] developed, for non-human primates, a semiautomated image segmentation method. A region of interest was drawn manually around the liver, then, based on T2* value, an automated algorithm was applied to segment the liver between liver parenchyma and islets clusters [32]. We recently developed a novel quantification method using the difference of two simultaneously acquired MRI images (three-dimensional ultra-short echo time) giving a positive contrast from the SPIO-labeled islet with suppression of the liver background and vessels, than can be confused with hypointense islets clusters with classical T2* sequences. An intensity threshold applied within the liver provides a number of enhanced pixels, referring to the number, but also to the size of the islet signal. Excellent correlation between signal intensity and number of transplanted islets was obtained [33•].

In Vivo Studies in Syngeneic Transplantation

With human Feridex-labeled islets, Evgenov et al. [26] showed that in immunodeficient mice, the number of islets decreased gradually during the first 2 weeks (–40%), suggesting a significant loss after islet injection, confirmed by graft TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) analysis showing high rates of apoptosis during the first days after transplantation. In the syngeneic primate model, the same team observed a 25% drop in transplanted islet mass between days 3 and 8, followed by stabilization of the graft up to day 30 [32]. In the rat model with Resovist-labeled islets by Kriz et al. [27], the MRI signal in the syngeneic group was unchanged between weeks 1 and 6, but in their pilot study in humans, a 45% signal loss was reported in 4 patients, 1 week after islet transplantation [31•]. In that study, the purity of the islet preparation was not reported and the first MRI was done 1 day after transplantation. It is probable that the loss of the signal was due in part to the loss of islet cells, but also to the loss of labeled exocrine cells between day 1 and week 1. Long-term studies to assess the permanence of the signal in syngeneic intraportal islet transplantation models are lacking.

Studies in Immune Rejection

SPIO nanoparticles have been studied in immune rejection models. Evgenov et al. [26] showed that the signal generated by Feridex-labeled human islets decreased faster and to a higher extent in immunocompetent mice than in NOD-scid mice, especially after 10 days, with increasing numbers of apoptotic cells seen in the graft. In this work,

histologic analysis showed that iron was detected in islet cells and not in infiltrating cells. At the time of islet-cell death, the iron was found in Kupffer cells, known for clearing rapidly free iron particles. Using Resovist in the allogeneic diabetic rat model, the Prague team also observed a 50% signal reduction at 2 weeks compared with the initial image at 1 week, contemporaneous with graft mononuclear cell infiltration [27]. In this study, based on glycemia, islet function was lost in all rats by day 12.

Studies in Humans

Two pilot studies reported MRI imaging of Resovist-labeled transplanted islets in patients with type 1 diabetes. With 1.5-T MRI imaging in four patients with type 1 diabetes, we were able to identify iron-labeled islets as hypointense spots within the liver [34]. Notably, all patients became insulin-independent, confirming the absence of islet cell toxicity by SPIO labeling. One participant had evidence of spontaneous liver iron overload (hemosiderosis), preventing islet visualization. A diffuse hypointense signal was observed in another patient after he had received intravenous iron therapy. At 3T, Saudek et al. [31•] could also visualize SPIO-labeled islets in the liver of seven patients from a group of eight. In four patients, the signal has been followed during 24 weeks and showed an important decrease of 45% in the first week followed by a much slower decline in the following weeks with a positive C-peptide measurement. However, the number of detected islets was still low taking into account the total number of infused islets, without obvious correlation between the number of visualized and transplanted islets.

Currently, studies on SPIO nanoparticles in islet transplantation have shown that islets can be labeled and visualized with clinical-grade MRI without toxicity and can be quantified by semiautomated analysis. Although signal persistence for more than 4 months was reported [25], the long-term stability of staining has not been fully explored in syngeneic models and the usefulness of this technique in the diagnosis and treatment of immune rejection has not yet been demonstrated. New SPIO nanoparticles are under development [35].

Ferromagnetic Iron Oxide Nanocubes

Recently, Lee et al. [36•] developed ferromagnetic iron oxide nanocubes (FIONs) that are polyethylene glycol-phospholipid-encapsulated magnetite nanoparticles of 58 nm. These nanocubes have shorter T2 relaxation time compared with Feridex and can label islets after overnight incubation without toxicity. FION-labeled islets could be observed as hypointense spots in the liver of syngeneic rat up to 150 days, whereas allogeneic islets disappeared within 15 days.

Gadolinium-Labeled Islets

Biancone et al. [37] used gadolinium-HPDO3A as an islet-labeling agent, providing islet hyperintense signal in a T1-weighted sequence. Although isolated islets could be seen in the liver of SCID mice, labeling did not persist beyond 60 days.

Islet Encapsulation Imaging

Islet encapsulation has been shown to protect islets against alloimmune rejection. Barnett et al. [38] added labeling agents inside the islet capsule. They first used a magnetocapsule (encapsulation of human islet and Feridex), transplanted intraportally in a swine model [38]. They showed that the hypointense signal could be easily detected in the swine liver at 3 weeks with significant human C-peptide levels. In a recent study, the same group used a fluorocapsule composed of encapsulated islets with perfluorocarbon, which can be detected under the swine kidney capsule using fluorine-19 MRI, but also ultrasonography and CT imaging [39]. However, with these techniques, islet viability and functionality cannot be correlated to the signal because the capsule can be detected whether or not the islets are alive.

Manganese-Enhanced MRI

All these techniques require *in vitro* labeling before transplantation and, therefore, the correlation between the islet mass and the functionality depends on the duration of label retention by the islets and rapid clearance after islet loss. Another way would be to enhance islets with a contrast agent injected during imaging that would reflect islet mass and islet functionality. In this view, manganese (Mn), a T1 contrast agent, represents a very interesting option, because in β cells, Mn^{2+} mimics Ca^{2+} influx preceding insulin secretion in response to hyperglycemia. Mn-enhanced MRI has shown that the signal coming from native pancreas of nondiabetic mice was increased by 51% after intravenous glucose load compared with saline load [40]. This increase was not observed in high-dose STZ mice and was only 20% in low-dose STZ mice. Mn-enhanced MRI seems to be a promising approach to evaluate β -cell functional mass in native pancreas. However, this technique might be difficult to apply to liver islet transplantation due to high Mn liver uptake and little signal coming from diffusely scattered islets.

Islet Vascularization MRI

MRI has also been tested to study islet vascularization. Hathout et al. [41] described dynamic contrast-enhanced imaging (DCE-MRI) to evaluate islet neovascularization

after intravenous injection of gadolinium–diethylenetriamine-penta-acetic acid. The rate of diffusion from the vasculature to interstitial spaces depends on microvessel surface area, permeability, and blood flow. Using a 11.7T rodent MRI studying mice after intraportal syngeneic islet transplantation, they showed a trend toward enhancement of the signal in the right liver lobe, which had received the islet transplantation, compared with the median and left lobes [42]. This was observed at day 7 but not at day 3, with a good correlation between the signal enhancement and neovascular density determined by von Willebrand factor immunohistochemical analysis. Using this technique in a marginal mass model with diabetic mice, the same authors found a negative correlation between the signal enhancement and the glycemia at day 14 [43]. However, this method has been described with high field rodent MRI in a selective intraportal transplantation model allowing comparison between grafted and ungrafted liver lobes to see the islet-based contrast enhancement. Moreover, the DCE sequence is done on a single slice and does not cover the whole liver, with a total acquisition time of 32 min, making human clinical application as yet unpractical. However, alterations in the microvasculature may reflect leukocyte infiltration and inflammation, as demonstrated in MRI analysis of NOD mice pancreas [44].

Positron Emission Tomography Imaging

Positron emission tomography (PET) is a noninvasive functional imaging technique that provides high resolution, good sensitivity, and accurate quantification of physiologic, biochemical, and pharmacologic processes in living subjects. This technique depends strongly on the choice of the radioactive probes.

^{18}F -Fluorodeoxyglucose

Earlier studies in islet transplantation used the classic tracer ^{18}F -fluorodeoxyglucose ($[^{18}F]FDG$), a glucose analogue that can be taken up by β cells. However, because the liver uptake is very high, this tracer cannot discriminate islets in the liver if injected intravenously before imaging. Our group was the first to use this marker by incubating *in vitro* the islets before intraportal transplantation in a rat model [45]. Using this technique in humans by labeling 23% of the transplanted islets, Eriksson et al. [46], showed that the radioactivity peak occurred 19 min after infusion was 75% of what was injected, illustrating the early loss that occurs during the transplantation procedure [46]. Distribution in the liver was heterogeneous with wide variation of concentration and location. However, because of the short half-life of ^{18}F (110 min), this technique can be used only to study islet survival and engraftment immediately after islet infusion.

Noninvasive Reporter Gene Probes Imaging

With this technique, a cell is transfected with a reporter gene to produce a specific protein that can be detected by the administration of a reporter gene probe. In 2006, two groups transfected human islets with adenovirus to express the herpes virus simplex 1 thymidine kinase (HSV1-TK) [47, 48]. After transplantation in a mouse model, [¹⁸F] FHBG, a substrate of HSV1-TK, was administered to quantify its retention in islets by microPET imaging. After axillar or subcapsular transplantation, the number of injected islets correlated well with the PET signal. But for intraportal transplantation, as the probe was cleared through the intestine and kidney, it gave spillover background signal in the liver of small animals. However, after transplantation, the correlation with islet number was good [48]. The main issue was that the PET signal returned to baseline after 30 days because of regression of expression of the reporter gene. By using a lentivirus vector, Lu et al. [49] prolonged graft monitoring up to 90 days. However, these techniques require pretransplant gene modifications possibly damaging islet cells, with limited direct human clinical applications.

Glucagon-like Peptide-1 Receptor PET Imaging

Because the glucagon-like peptide-1 (GLP-1) receptor is highly expressed in islets, GLP-1 receptor ligand could be an ideal probe for PET imaging of islets. Recent studies showed that labeled exendin holds great potential for noninvasive imaging of β cells in the native pancreas [50••] and can be useful in humans for detection of pancreatic insulinoma [51, 52•]. In the latter study, [111In] DOTA-exendin-4 successfully detected insulinoma in all six patients, with high kidney uptake, but very low liver background, suggesting potential application for intraportal islet transplantation. With this technique, Pattou et al. [53] recently reported successful imaging of a functioning islet autograft in the forearm muscle of the recipient.

The Quest for In Situ β -Cell Labeling

The development of PET or single photon emission computed tomography probes able to label β cells in situ after systemic administration is a favored research area. They would provide a method that can be repeated over time to visualize islet grafts, but could also be useful to monitor islet mass within the pancreas in type 1 diabetes [54]. The main problem of this approach is the specificity and affinity of the marker used to label islet cells. Studies performed by Sweet et al. [55] calculated that the specific affinity for a cell-labeling agent should be over 1,000 times higher than that for the surrounding tissue for the imaging

of islets within the pancreas; this figure rises to 5,000 for islets transplanted into the liver. Although the [111In] DOTA-exendin-4 probe, developed for insulinoma imaging, was able to faintly image intramuscular islet autografts [46•], affinity issues have not yet rendered in situ labeling applicable for intraportally transplanted islets.

Conclusions

Although in vivo imaging of transplanted islets is a rapidly developing field, correct evaluation of transplant β -cell functional mass remains a problem. The majority of studies highlighted in this review used an islet labeling agent before transplantation, with some issues. First, in vitro preinfusion manipulation is required, potentially altering islet functionality. Second, long-term persistence of the labeling in functioning islets is difficult to obtain. Third, the detected signal does not reflect directly the islet function, potentially inducing false-positive results. Relevant methods showing promise in animal models are difficult to translate to clinical practice: imaging of islet transplant under the kidney capsule is totally different from imaging islets scattered throughout a whole human liver, and high-field-strength systems used in rodents have higher spatial resolution than low-field-strength clinical-grade systems.

The development of safe and specific tracers of islet graft function and mass is an important objective for further studies. Multimodal imaging techniques such as combined PET and MRI may be promising tools for clinical islet transplantation research with high spatial resolution and sensitivity. However, the low availability of such imaging methods make them as yet difficult to use as routine and systematic monitoring for islet transplant recipients.

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- Of importance
- Of major importance

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