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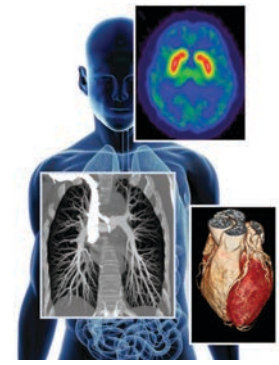

Imaging in Medicine

Theranostic MRI: the future for Type 1 diabetes management?

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Diabetes mellitus is the most common metabolic disorder worldwide. Type 1 diabetes mellitus results from the lack of insulin production induced by autoimmune destruction of pancreatic β-cells. Theranostic MRI is an emerging field that uses nanometer-scale materials to provide diagnostic insight with simultaneous treatment. These nanoparticle platforms can accommodate targeting ligands, therapeutic moieties, along with complementary imaging modalities for targeted therapeutic goals, while achieving highly accurate multimodality imaging. In this review, we summarize various types of nanocarriers that have been explored for theranostic imaging. We also outline recent progress in theranostic MRI for Type 1 diabetes management. Finally, we discuss future considerations and opportunities afforded by theranostic imaging as a new platform in this field.

Keywords: diabetes • islet transplantation • molecular imaging • MRI

Type 1 diabetes (T1D), which accounts for an estimated 5–10% of diabetic Americans, is a chronic and potentially disabling disease that represents a major public health and clinical concern. T1D is an autoimmune disease in which $CD4^+$ and $CD8^+$ T cells infiltrate the islets of Langerhans, resulting in β-cell destruction, leaving patients dependent upon exogenous insulin for survival [1]. Despite advances in diabetes technologies and therapeutics, the majority of T1D patients do not reach the recommended glycemic goals and remain at high risk for developing microvascular complications [2]. Insulin has been proven to control hyperglycemia and delay progression of some complications [3]. However, a clear need exists to develop novel treatments that can either affect the underlying pathophysiology of the disease and/or help patients with established disease to improve glycemic control [4].

Theranostic imaging is a rapidly growing branch of medicine nourished by the progress achieved in the diverse areas of material science, synthetic chemistry and molecular imaging.

The term 'theranostic' is defined as a material that combines the modalities of therapy and diagnostic imaging [5]. In contrast to the development and use of separate materials for these two objectives, theranostics combine these features into one 'package', which has the potential to overcome undesirable differences in biodistribution and selectivity that currently exist between distinct imaging and therapeutic agents [6–10].

MRI does not utilize ionizing radiation, has tomographic capabilities, can deliver the highest resolution images *in vivo* and has unlimited depth penetration [11]. The contrast used for MRI originates from local variations of water protons and the chemically bound state of proton concentrations in tissues [12]. Two relaxation time constants, including spin–lattice relaxation (T_1) and spin–spin relaxation (T_2), associated with the time decay of proton magnetization back to equilibrium following a radio-frequency pulse, are used to measure intrinsic tissue variations that generate anatomical contrast [13]. Since

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MRI is not sensitive enough for early diagnosis, development of the novel MRI agents for achieving higher sensitivity is equally important. Magnetic nanoparticles are among the most widely utilized contrast agents for MRI. Not only can magnetic nanoparticles be functional as imaging tools, but they can also be useful as a therapeutic moiety carrier and an actuator for controlled drug release, which potentially makes MRI one of the most important modalities for the application of theranostic imaging [14].

During recent years, considerable efforts have been devoted to the development of the theranostic MRI for T1D and islet transplantation. This current article summarizes the latest developments in various theranostic MRI probes designed for T1D treatment and management.

Theranostic nanocarriers as smart actuators for MRI

Multidisciplinary field of theranostic imaging combines molecular biology, synthetic chemistry and nanotechnology. Nanoparticles used for imaging are generally categorized as organic and inorganic materials. Organic nanoparticles include polymers, polymeric micelles and dendrimers. They are developed primarily for drug delivery [14]. Compared with organic nanoparticles, inorganic platforms posses more diverse and distinct physical features contingent to their size and composition. Of particular significance is the use of magnetic nanoparticles for theranostic MRI. The ease of the synthesis of magnetic nanoparticles and subsequent surface modifications to introduce additional therapeutic and targeting functionalities has enabled these systems to be employed as a smart platform for the MRI. The composition and size of these nanometer-sized materials enable multiple components to be carried. Various cell-specific imaging, targeting and therapeutic moieties can be incorporated into a single magnetic nanoparticle that is designed for theranostic MRI [15,16].

Imaging reagents used for theranostic MRI

The ideal theranostic MRI probes should be able to:

- Selectively accumulate in target tissues or cells;
- Effectively deliver therapeutic moieties;
- Provide morphological and functional information of the area;
- Biodegrade with safe byproducts [7].

Although no probe has achieved all of the above criteria, some of them possess one or more features. To date, the probes applied in theranostic MRI include iron oxide nanoparticles, gadolinium (Gd)-loaded nanoparticles and manganese (Mn)-based nanoparticles.

Iron oxide nanoparticles for theranostic MRI

Superparamagnetic iron oxide (SPIO) nanoparticles used for theranostic MRI are usually composed of three components:

- A biodegradable superparamagnetic iron core for MRI;
- A polymer surface coating, that not only serves as a protective layer, but also as a functional layer decorated with various linkers;
- Various functional moieties attached to the coating that serve as targeting macromolecules, therapeutic payloads or additional imaging tags (Figure 1) [14,17].

The iron oxide core affects the transverse (spin–spin) relaxation time of protons in nearby tissues. It decreases the T_2 relaxation time, which can be efficiently visualized as a darkening of the tissues on T_2-weighted MRIs. There have been several coating materials utilized for stabilizing the particles, controlling their size and transforming them into multifunctional nanodrugs, such as polyethylene glycol (PEG), dextran, silica, polyethylenimine, polyvinylpyrrolidone, fatty acids, polypeptides, chitosan and gelatin, among others [17]. Iron oxide nanoparticles are clinically approved under many brand names including Resovist® (Schering AG, Germany), Ferumoxtran-10 (Sinerem®, Guerbet, France; Combidex® Advanced Magnetics Inc., MA, USA), Gastromark® (AMAG Pharmaceuticals, Inc., MA, USA) and Feridex® (AMAG Pharmaceuticals, Inc., MA, USA).

Iron oxide magnetic nanoparticles are useful for visualizing the location and trafficking of therapeutic moieties *in vivo*. It is also possible to encapsulate these nanoparticles with drugs in a nanogel for monitoring drug release in the target areas. Zhang *et al.* developed multifunctional and degradable nanogels encapsulating both model drug (fluorescently labeled dextran) and iron oxide nanoparticles by polymerizing zwitterionic monomers with a disulfide crosslinker. This theranostic nanogel exhibited targeted delivery to human umbilical vein endothelial cells. Once the nanogels enter the reducing intracellular zone, the disulfide bonds are cleaved thus releasing the sample drug, which could be imaged by MRI [18].

Renal excretion represents an important route of elimination for circulating SPIO nanoparticles. Studies have demonstrated that SPIO nanopaticles with dextran coating underwent nearly 25% elimination via the urine and feces over a period of 19 days in animals [19]. The internalized iron oxide nanoparticles get

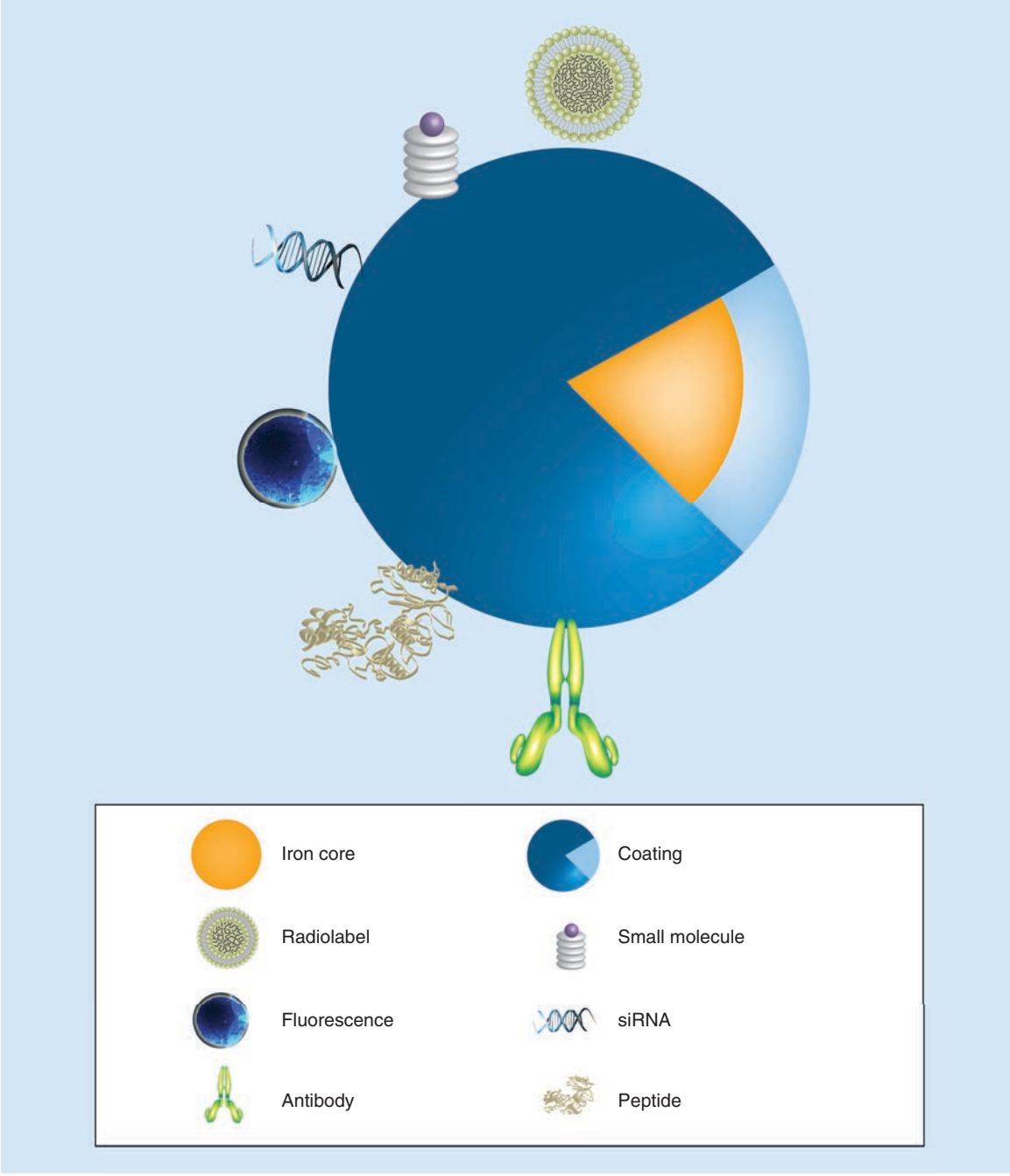

Figure 1. Theranostic magnetic nanoparticles consist of magnetic core/cores surrounded by a coating that is complexed with various moieties for additional functionalities.

degraded at low lyzosomal pH, releasing free Fe(III) into the cytoplasm. The released iron is stored in the body reserves with the help of iron-regulating proteins such as ferritin and hemosiderin [20]. Particle size, surface coating and surface charge are major determinants of the biodistribution and pharmacokinetics of SPIO nanoparticles [21].

SPIO nanoparticles, as a negative contrast agent, has certain shortcomings. T_2- and T_2*-weighted images generally provide lower resolution compared with T_1-weighted images. In some cases, owing to their negative contrast, it is difficult to distinguish iron oxide nanoparticles from other hypointensities in the MRI, which may arise from vascular hemorrhage or iron-rich tissues, such as the spleen and liver [22,23].

Gd-loaded nanoparticles for theranostic MRI

Gd-based contrast agents are paramagnetic contrast agents that mainly reduce T_1 relaxation time resulting in positive (brighter) contrast in T_1-weighted

images [24]. The most commonly used Gd-based agents are usually obtained by compexation of the Gd^{3+} ion with chelates, such as diethylene-triamine-pentaacetic acid, 1,4,7,10-tetraazacyclo odecane-1,4,7,10-tetraacetic acid and di-pyridoxyl-di-phosphate [25,26]. Gd complexes can also be conjugated to different enzymes or ligands for theranostic MRI. Major classes of Gd-loaded nanoparticles include lipid-based nanoparticles, polymeric nanoparticles, micelles, dendrimers and Gd–silica nanoparticles [9].

Tse *et al.* prepared Gd and europium-doped silicate nanoparticles that act as bimodal imaging agents for MRI and luminescence. The longitudinal relaxivity and transverse relaxivity, a measure of MRI contrast agent efficiency, were up to four-times higher than that of the clinically employed Omniscan™ (gadodiamide; GE Healthcare Inc., WI, USA). In addition, these mesoporous nanoparticles have the potential to serve as controlled release matrices for theranostic imaging [27].

Gd-based contrast agents have also been applied for encapsulation for cell tracking and theranostic MRI. Arifin *et al.* introduced a biohybrid theranostic agent, in which Gd-based nanoparticles served as trimodal diagnostic markers for cell transplantation monitoring. The grafts were successfully localized with T_1-weighted MRI. By placing the contrast agent formulation in the extracellular space of the hydrogel, large amounts of contrast agents were incorporated with negligible toxicity. Besides the diagnostic function, the encapsulation also protected the grafts from immune rejection [28,29].

A potential issue in this approach is that free Gd ions are cytotoxic and could be retained in the liver, spleen and other organs. Gd-based contrast agents have been associated with nephrogenic systemic fibrosis in patients with severe renal diseases [30].

Mn-based nanoparticles for theranostic imaging

Mn has paramagnetic properties with five unpaired electrons that permit a high spin number, long electronic relaxation times and labile water exchange [31]. Mn-based contrast agents can be classified into two types: small molecule agents and macromolecular agents. Small molecule agents are, similar to Gd chelates, complexes of Mn^{2+} ions with chelates such as di-pyridoxyl-di-phosphate or diethylene-triamine-pentaacetic acid. Macromolecular agents consist of Mn oxides such as MnO, MnO_2 and Mn_3O_4 [32]. These oxides can be formulated into nanoparticles, which could be functionalized for theranostic purposes [26,33].

Bae *et al.* synthesized multifunctional hollow MnO nanoparticles by a bioinspired surface functionalization approach, using 3,4-dihydroxy-L-phenylalanine (DOPA) as an adhesive moiety, for targeted delivery of therapeutic gene silencing and simultaneous MRI monitoring [34]. Howell *et al.* reported the design and synthesis of multifunctional lipid–micellar nanoparticles containing MnO for theranostic MRI. Oleic acid-coated MnO nanoparticles were encapsulated in micelles composed of polyethylene glycol, phosphatidylethanolamine, cholesteryl 3β-*N*-(di-methyl-aminoethyl)-carbamate hydrochloride and dioleoyl-phosphatidylethanolamine. The particles were loaded with plasmid and could be efficiently taken up by target cells *in vivo*. These results demonstrate that Mn-based nanoparticles are capable of theranostic MRI [31].

Although these proof-of-concept studies demonstrate the exciting advances in preparing Mn-based nanoparticles, some issues including cytotoxicity still need to be considered. Studies show that the brain is vulnerable to Mn exposure. Symptoms comparable to the characteristics of Parkinson's disease have been reported [33].

Therapeutic & target moieties for theranostic MRI

Advances in nanoparticles technology have created new paradigms for theranostics imaging. Simultaneous-targeted imaging and therapy are made possible by attaching a variety of imaging and therapeutic components [14]. Nanoparticle vehicles can accommodate a wide variety of drug candidates that have been shelved owing to solubility or pharmacokinetic reasons. In addition to small molecules, other moieties such as siRNAs, DNAs and peptides can effectively be delivered via these carrier platforms [14,16].

siRNAs

siRNAs are short double-stranded RNA containing 19-23 nucleotides. They can suppress a target complementary gene at the post-transcriptional mRNA level by means of RNAi, which is a promising therapeutic tool for a variety of diseases including diabetes. So far, the delivery of naked siRNAs is hindered by the non-specific distribution, poor cellular uptake and the failure of endosomal escape [35]. Nanoparticles have several favorable attributes such as uniform size, superior biocompatibility and facile surface modification that qualify them as candidates for siRNA *in vivo* delivery tools. At the same time, magnetic nanoparticles can serve as a real-time imaging agents for monitoring siRNA transport into the cell and subsequent release at the target site. For *in vivo* delivery, siRNA is conjugated to the nanoparticles via disulfide linkage that can be cleaved enzymatically for its facile release. Subsequent endocytosed nanoparticles escape from the endosome, glutathione breaks the disulfide bond to release the siRNA for the inhibition of protein expression [36].

DNA

Biological molecules such as DNA represent the genetic material of most organisms and organelles. Most hereditary information is encoded in the chemical language of DNA and reproduced in most cells of living organisms [37]. The double-stranded helical structure of DNA is a key to its use in applications of replacing a mutated gene causing diseases with a healthy copy of the same gene.

Bhakta *et al.* developed Gd oxide-doped silica nanoparticles (50 nm), the surface of which was functionalized to anchor DNA. The surface of the nanoparticle was modified by 3-aminopropyltrimethoxysilane, which allowed for electrostatic binding of DNA. The plasmid DNA held over the surface of the nanoparticle was firmly immobilized and protected from DNase attack. These particles are paramagnetic with high transfection efficiencies in cells *in vitro* [38].

Chen *et al.* developed a nanoplatform that effectively transports plasmid DNA into T cells by attaching a T-cell specific ligand, the CD3 single-chain antibody, to the ends of PEG-grafted polyethylenimine. This polymer was first complexed with SPIO nanoparticles and was subsequently used to condense plasmid DNA into nanoparticles. The reporter gene assay demonstrated that the nanoplatform functionalized with a targeting ligand, produced a 16-fold increase in gene transfection in a T-lymphocyte cell line. In addition, MRI successfully visualized this targeting event in cell culture [39].

As a versatile gene vector, minicircle DNA (mcDNA) has a great potential for gene therapy. However, some serious challenges remains, such as effective delivery of mcDNA into targeted cells/tissues and noninvasive monitoring of mcDNA delivery. Wan *et al.* developed an MRI visible gene delivery system with a core consisting of SPIO nanocrystals and a shell made out of biodegradable stearic acid modified with low-molecular-weight polyethyleneimine via self-assembly. Furthermore, the nanoparticle could effectively bind with mcDNA and protect it from enzymatic degradation [40].

miRNA

miRNAs are small noncoding RNA molecules consisting of 21–23 nucleotides that regulate gene expression by targeting mRNAs for either cleavage or translational repression [41]. They have been shown to play important roles in a broad range of biological processes including development, cellular differentiation, proliferation and apoptosis by association with the 3′ untranslated region of target mRNAs [42–44]. It is now becoming clear that miRNAs make an important contribution to alterations in gene expression observed in dysfunctional β-cells and are likely to be involved in the development of T1D [45]. On the other hand, miRNAs could play an important role in T1D therapy. For example, Cantaluppi *et al.* [46] reported that microvesicles released from endothelial progenitor cells could enhance human islet vascularization by carrying proangiogenic miR-126 and miR-296 targeting islet endothelium. The development of theranostic imaging techniques could assist in delivering of miRNA mimics or anti-miRNA nucleotides to correct the level of key miRNAs under diabetic conditions and lead to new strategies for treating T1D [47].

Schade *et al.* developed a technique to efficiently deliver miRNA into bone marrow-derived human mesenchymal stem cells with the help of a magnetic nonviral vector based on cationic polymer polyethylenimine bound to SPIO nanoparticles. This study shows high potential of theranostic imaging in stem cells transplantation for diabetes treatment [48].

Here, we summarize contrast agents and therapeutic moieties that appeared to be promising for the theranostic imaging of T1D. These tools have to be tested further for T1D specific pathological targets.

T1D pathological targets for theranostic MRI

T1D is an autoimmune disease characterized by complex lymphocytic infiltrate accompanied by a range of alterations in the microvasculature, culminating in specific destruction of insulin-producing β-cells [49]. Microvasculature leakage, mononuclear cell infiltration of the pancreatic islets and β-cell destruction are the pathological hallmarks of T1D [50–52]. New strategies, involving theranostic imaging, have been designed to target these biomarkers for the purpose of prevention and therapy.

Imaging of vasculature changes during the progression of inflammation

The progression of inflammation is associated with changes in pancreatic islet vasculature and subsequent vasculature dysfunction. At sites of inflammation, blood vessels become 'leaky' and allow large molecules to extravasate through the walls of the damaged vessel into the surrounding tissue. This process of passive accumulation of nanoparticles at sites through leaky vasculature, is known as the enhanced permeability and retention effect, which is sufficient to favorably alter biodistribution in order to improve the diagnostic and therapeutic efficacy [16]. Vascular leakage could be utilized for delivering therapeutic and imaging agents to the islets. Our group demonstrated that this property of islet vasculature could be exploited for delivery of protected graft copolymer (PGC) labeled with Gd diethlene-trimine-pentaacetic

acid and fluorescein, a T_1 contrast blood pool agent. *In vivo* MRI was used to demonstrate accumulation of this agent in the areas of leaky vasculature in the islets of streptozotocin (STZ)-induced diabetic mice [53]. Subsequently, the same contrast agent was utilized to assess changes in islet vasculature in a spontaneous T1D animal model. It was demonstrated that PGC labeled with Gd diethlene-trimine-pentaacetic acid and fluorescein, could highlight changes in vascular permeability and blood volume of diseased pancreatic islets [54].

For therapeutic applications, Castillo *et al.* utilized fatty acid-containing PGC for delivery of GLP-1 to pancreatic β-cells for treatment of Type 2 diabetes [55]. GLP-1 is a hormone that inhibits β-cell apoptosis, restores glucose sensitivity and stimulates β-cell proliferation and differentiation [56]. Native GLP-1, which is cleaved by DPP-4, has a very short blood half-life. In the above study, PGC was used to stabilize GLP-1, prolonging its blood half-life and, ultimately, delivering it to pancreatic islets. *In vivo* data proved higher efficacy of PGC-GLP-1 in maintaining blood glucose levels in diabetic male Zucker fatty rats compared with exendin-4 [55]. This study indicates theranostic potential of a long circulating blood pool agent for diabetes treatment.

Superparamagnetic T_2 contrast agents have also been exploited for visualization of the changes in the microvasculature that invariably accompany inflammation. SPIO nanoparticles were used for detection of vascular leakage in association with insulitis in murine models of T1D, permitting noninvasive visualization of the inflammatory lesions in real time [57,58]. Pushing this work forward to clinic translation, Gaglia *et al.* reported on the development of a MRI method to visualize active insulitis in T1D patients. Ten patients and 12 healthy controls received T_2 contrast agent SPIO (ferumoxtran-10) at a dose of 2.6 mg of iron/kg of bodyweight over a period of 30 min. MRI analysis of the pancreas on three consecutive slices demonstrated lower T_2 relaxation time within the pancreas at 48 h, suggesting higher retention of the probe in the patient compared to control subjects. They also found that the patient had more heterogeneity in T_2 throughout the pancreas with a greater change in T_2 in the head and body compared with the tail of the pancreas [59]. The authors believe that these SPIO particles could migrate from the leaky vessels into the surrounding tissue, where they are phagocytized by inflammatory cells [59]. The road to the above studies was paved in earlier publications describing labeling of these inflammatory cells with magnetic nanoparticles for the purpose of detection of early insulitis [60,61]. These studies are discussed in more detail below.

Theranostic imaging of the lymphocyte infiltration during the progression of inflammation

Chronic infiltration of islets by autoreactive T cells, results in the destruction of insulin-producing β-cells, leading to the onset of T1D. Therefore, most therapeutic strategies target lymphocyte populations responsible for this destruction [62]. Development of noninvasive imaging techniques for tracking infiltrating T cells *in vivo* could aid in early diagnosis and treatment of diabetes [10]. Several studies have already demonstrated the potential of *in vivo* MRI for tracking labeled cytotoxic T lymphocytes infiltrating pancreatic islet in different animal models (Figure 2) [60,61,63,64].

To selectively image T cells involved in autoantigen recognition, antigen-specific SPIO nanoparticles were synthesized by Medarova *et al.* [65]. They consisted of iron oxide nanoparticles conjugated through avidin–biotin linkage to a complex of MHC with NRP-V7 peptide (a high-avidity mimotope of IGRP) [49,66]). Subsequent to intravenous injection in nonobese diabetic (NOD) mice of different age, the target-specific nanoparticles recognized H-2Kd-restricted $CD8^+$ T cells infiltrating pancreatic islets. As determined by MRI analysis of pancreas-associated T_2 relaxation times, accumulation of the nanoparticles in the pancreata was age-dependent, correlated well with the progression of insulitis and provided quantitative information on the infiltration of $CD8^+$ T cells carrying TCR specific for NRP-V7 peptides. This study indicated that this approach could serve as a surrogate marker for selecting T1D patients who might benefit from immunosuppressive therapy at the early stage of the disease. Having proved accumulation of target-specific nanoparticles in antigen-specific T-cells, we subsequently investigated the utility of these complexes for theranostic purposes. Studies in NOD mice demonstrated that accumulation of nanoparticles coated with disease-relevant peptide–MHC complexes led to protection from and reversal of diabetes in these animals by expanding (in an epitope-specific manner) a subset of antigen-experienced autoreactive $CD8^+$ cells (Tregs) that suppressed the activation and recruitment of noncognate specificities to islets [67]. This study demonstrated that target-specific nanoparticles have the potential to become powerful theranostic tools for diabetes treatment [67].

Development of theranostic probes for targeting endogenous pancreatic β-cells

The availability of β-cell-specific imaging agents is of critical importance for advancement of our understanding and treatment of T1D [68]. To date, approaches that have been explored for developing

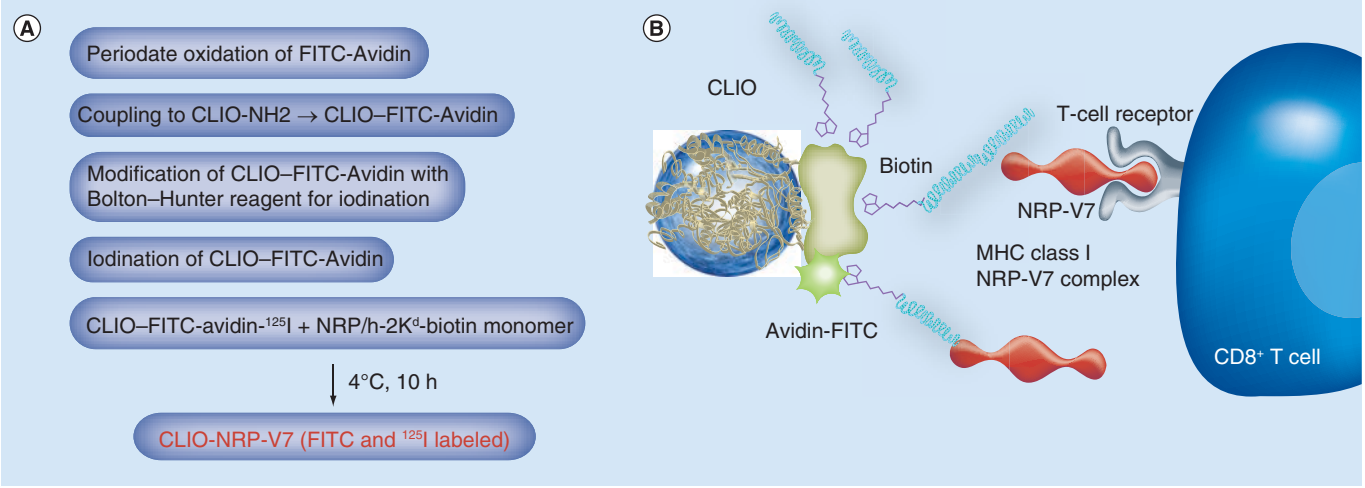

Figure 2. Magnetic nanoparticles designed for MRI tracking the recruitment of diabetogenic CD8⁺ T cells to the pancreas. (A) Step-by-step synthesis of CLIO-NRP-V7 probe. **(B)** Schematic representation of CLIO-NRP-V7 probe. Biotinylated NRP-V7–H-2K^d complex was coupled with CLIO particles modified with avidin. CLIO-NRP-V7 probe is recognized by the T-cell receptor on NRPV7-reactive CD8⁺ T cells. Note that there are four binding sites for biotin on avidin and, hence, for biotinylated peptide–MHC complex.
CLIO: Cross-linked iron oxide; FITC: Fluorescein isothiocyanate; NRP: Nonobese diabetic-relevant peptide.
Reproduced with permission from the American Diabetes Association [60].

β-cell-specific MRI probes included Mn-enhanced MRI [69], zinc-responsive T_1 agent [70] and GLP-1 receptor (GLP-1R)-targeting iron oxide nanoparticles [71]. Among these probes, GLP-1R-targeting nanoparticles seem to be one of the candidates for theranostic MRI of endogenous islets.

Exploiting the fact that GLP-1R is highly expressed on β-cells [72], Zhang *et al.* developed targeted SPIO nanoparticles using GLP-1 analog exendin-4 as a ligand. The results demonstrate that exendin-4 conjugated nanoparticles specifically bind to and internalized by GLP-1R-expressing β-cell line [71]. Notably, systemic delivery of exendin-4-loaded magnetic nanoparticles in nude mice bearing insulinomas, led to generation of a strong MRI contrast. While these results opened the door for theranostic MRI of the native islets, they were limited to imaging insulinomas. Clearly, more studies are required to prove that imaging of endogenous islets would be possible by targeting GLP-1R, considering that this receptor is also expressed in other tissues [73]. Keeping in mind that GLP-1 and its analogs have been used for treatment of both Type 1 and 2 diabetes [74,75], the potential of this theranostic approach cannot be underestimated.

Overall, the field of theranostic MRI of pancreatic islets has undergone a significant surge; however, it still suffers from limited resolution of the imaging modality and lower specificity of the probes targeting β-cells. At present, single-cell resolution cannot yet be achieved in order to enable differentiation between scattered islets, single β-cells or surrounding tissue. Imaging biomarkers should adhere to several strict requirements. The successful candidate:

- Should be expressed specifically by β-cells and not by any other pancreatic cells;
- Should be expressed in a sufficient number of copies to be available for an imaging probe;
- Tissues surrounding the pancreas should be devoid of this marker in order to avoid high background signal from these organs during imaging [76].

In an extensive review on the etiology, immunology and therapeutic strategies of T1D published in 2011 [77], it was stated that monoclonal IgM antibody IC2 [76], which specifically binds to the surface of β-cells, might be the only reliable marker for noninvasive imaging and quantification of native β-cells [77,78]. In line with previous work carried out by Kavishawar *et al.*, we have recently successfully identified the IC2 antigen [79], which revealed itself in a form of cholesterol-stabilized sphingomyelin patches on β-cells. These findings have significant implications for developing theranostic strategies for targeting endogenous β-cells.

Theranostic MRI in islet transplantation

Islet transplantation has emerged as one of the most promising therapeutic approaches for T1D treatment during the recent decade [80]. The Edmonton protocol significantly improves the short-term rate of success of islet transplantation, with an 80% insulin independence being at 1-year postallergenic islet transplantation [81]. Unfortunately, this rate decreases dramatically to 10% by 5 years after transplantation. Studies identified that a number of immunological and nonimmunological factors contribute to islet graft loss after transplantation [82,83].

The factors that underlie the deterioration of islet-graft function include allogeneic immune response [84], recurrence of autoimmunity [85], instant blood-mediated inflammatory reaction, hypoxia-induced cell apoptosis, and nonspecific inflammation [83]. Theranostic MRI is a promising approach that enables tracking of transplanted islets and detection of the islet graft injury combined with the means of graft protection.

Theranostic MRI for graft rescue

Beyond being functionalized as a tool for diagnostic imaging of islet grafts, contrast agents can also deliver therapeutic moieties. siRNAs are small molecules that are capable of selectively silencing the expression of any gene of choice with single nucleotide specificity [86]. Taking advantage of the propensity of pancreatic islets to avidly take up dextran-coated SPIOs, Medarova *et al.* designed a probe that consisted of a dextran-coated iron oxide core, conjugated to siRNA. The utility of this approach was investigated by delivering siRNA-nanoparticle probes, which target genes implicated in apoptosis, to islets prior to islet transplantation. As proof-of-concept, our studies firstly demonstrated that siRNA-tagged iron nanoparticles could accumulate in pancreatic islets in quantities sufficient for detection by MRI *in vitro* and for silencing target genes (*GFP* was used as a model gene) [87]. More recent studies by Wang *et al.* used a theranostic nanoparticle probe to target the apoptotic-related gene caspase-3 in islets prior to transplantation. MRI showed improved survival among protected islets compared with islets in the control group [88]. We then further boosted islet survival by using a theranostic nanoparticle probe, to silence MHC class 1 molecule β-2 microglobulin, a protein associated with the histocompatibility complex that is involved in T-cell recognition of β-cells. Silencing β-2 microglobulin enabled islet protection from immune rejection caused by adoptively transferred splenocytes. MRI analysis of graft volume revealed that, as expected, volumes of both experimental and control grafts decreased post T-cell transfer. However, the rate of graft volume decrease in the experimental group was significantly lower than in the control group, consequently resulting in a delay in hyperglycemia caused by T-cell challenge. As such, the mean time for developing diabetes in the control group was 6.5 ± 4.5 days, whereas in the experimental group it was delayed by up to 23.8 ± 4.8 days [89]. Theranostic probes used in these studies successfully served as an imaging reporter for monitoring graft fate and as a vehicle for therapeutic delivery [88,89]. These studies have laid the groundwork for future clinical translation, in which genes responsible for islet damage can be targeted by delivering a cocktail of nanoparticles, or nanoparticles decorated with various sets of siRNAs, in order to improve graft outcome. Furthermore, the inherent imaging capabilities of this approach permit the noninvasive tracking of the contrast agent conjugated to therapeutic moiety and its relationship to graft fate (Figure 3) [90,91]. Conceivably, this technique represents a valuable tool, not only for monitoring the disease onset and progression, but also for assessing the delivery of therapy using imaging-guided paradigms for future studies on T1D [92].

Theranostic imaging of encapsulated islet grafts

Another theranostic MRI strategy for transplanted islets is graft encapsulation with magnetocapsules. Barnett *et al.* developed SPIO-labeled alginate magnetocapsules for monitoring and immunoprotection of islet grafts [93]. These magnetocapsules proved their functionality by restoring normoglycemia in STZ-induced diabetic mice and in diabetic swine transplanted with human islets. In addition, MRI provided the ability to monitor distribution and localization of transplanted pancreatic islets over time *in vivo* in real time. Later, Barnett *et al.* included perfluorocarbon emulsions detectable by ^{19}F-MRI and ultrasound, into alginate islet microcapsules with encapsulated islets. The results showed that perfluorocarbons did not alter the permeability of the capsules or affect islet function [94]. Arifin *et al.* encapsulated human pancreatic islets with Gd chelate-loaded magnetocapsules. Grafts were functional *in vivo* and normoglycemia was sustained for at least 6 weeks without the use of immunosuppressive drugs [28]. These studies demonstrated exceptional theranostic capabilities of magnetocapsules for protecting transplanted grafts from immune rejection and for tracking islets with noninvasive imaging [95,96].

Early detection of immune rejection in transplanted islets

Damage, due to immunologic factors after transplantation, is one of the main reasons for islet graft failure in diabetic patients. Immune rejection contributes to long-term losses of the islet grafts. Current immune monitoring methods usually identify graft dysfunction when it is too late to take action [97]. A reliable monitoring tool is required in order to allow for earlier detection of harmful events. More importantly, detection of immune-mediated rejection at an early stage would facilitate prompt intervention. The studies by Wang *et al.* recently reported on an application of dual contrast-enhanced MRI for monitoring immune rejection in transplanted islet grafts at an early stage. In our studies, NOD/severe combined immunodeficiency mice were transplanted with SPIO-labeled human islets under the kidney capsule. To induce immune rejection, these mice were adoptively transferred with splenocytes

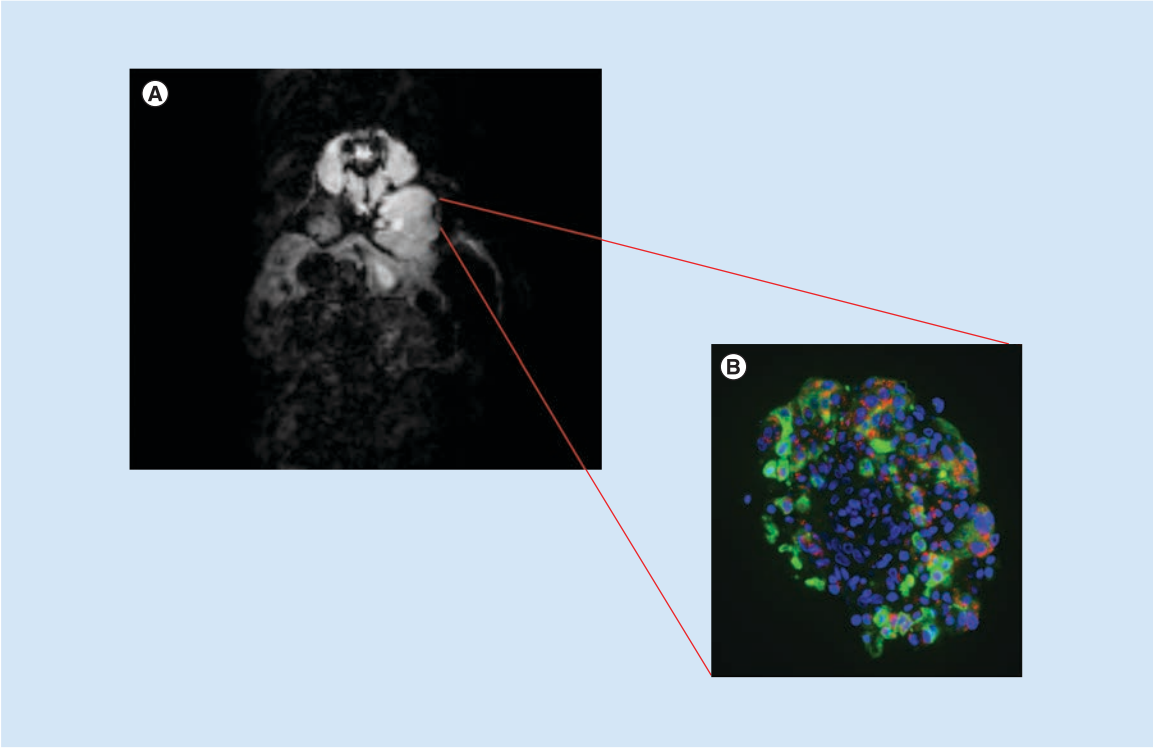

Figure 3. Theranostic imaging of the grafts transplanted under the kidney capsule. (A) T_2-weighted MRI of the graft labeled with iron oxide nanoparticles carrying siRNA to β-2 microglobulin. **(B)** Fluorescence microscopy of the labeled islet shows heavy labeling of islet cells with theranostic nanoparticles.
Green: Insulin stain; Red: Nanoparticles; Blue: Cell nuclear stain.
For color images please see online www.futuremedicine.com/doi/full/10.2217/iim.13.67

from NOD diabetic mice. To detect immune attack, we utilized protected graft copolymer labeled with Gd diethlene-trimine-pentaacetic acid and fluorescein (PGC-Gd-DTPA-F), which accumulates in the sites of inflammation characterized by enhanced permeability and retention effect. MRI results demonstrated significantly greater accumulation of PGC-Gd-DTPA-F in the graft area after adoptive transfer compared with that before the transfer. Graft area was identified by the signal loss due to the presence of iron oxide-labeled islets. These results were confirmed by histological staining, which showed notable leakage of the contrast agent into the islet cell interstitium. These results demonstrated that PGC-Gd-DTPA-F-enhanced MRI allows for *in vivo* assessment of vascular damage of the graft due to immune rejection (Figure 4) [98,99]. Considering PGC as a versatile carrier for *in vivo* drug delivery [100], this platform could be used for theranostic MRI of early detection and treatment of immune rejection of islet grafts in the future.

Outlook & perspective

Multimodality theranostic imaging for T1D

To date, no single imaging modality can fulfill all the requirements of specifically imaging β-cell mass and monitoring transplanted islets or inflammatory process involved in insulitis *in vivo*. Each imaging modality has its advantages and limitations in terms of sensitivity, tissue penetration, spatial resolution and clinical potential [13]. MRI has high spatial resolution and the highest soft-tissue contrast; however, it gives little functional and quantitative information [92]. By contrast, nuclear imaging including PET and single-photon emission CT can give information regarding the physiological status of the particular target organ. Disadvantages of nuclear imaging include low spatial resolution and tracers' short half-life time [68,101]. With rapid development of imaging facilities and multifunctional probes, combining complementary imaging modalities seems to be the solution of choice. Combined and simultaneous PET/MRI could provide the exact anatomical localization and the quantification information of the target organs in humans. With this new bimodal approach novel functional–anatomical and multiparametric applications become feasible that can be expected to deliver information beyond that accessible by separately applied modalities. Although the two technologies were initially regarded as inherently incompatible, different solutions have been developed and implemented to realize PET/MRI instruments for both small-animal and human-bimodal imaging [102]. In addition, novel

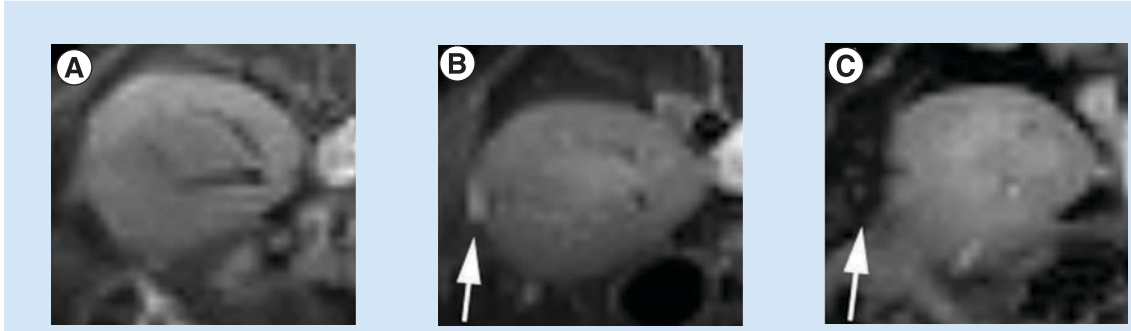

Figure 4. Dual-contrast MRI of rejection of islet cell grafts labeled with iron oxide nanoparticles. (A) T_1-weighted image of mouse with intact graft 17 h after injection of protected graft copolymer-gadolinium-diethylene-triamine-pentaacetic acid-fluorescein shows no enhancement in graft area. **(B)** T_1-weighted image obtained 17 h after injection of graft copolymer-gadolinium-diethylene-triamine-pentaacetic acid-F in animals that received adoptively transferred splenocytes, shows intense enhancement of graft area (arrow). **(C)** T_2*-weighted image of adoptively transferred animals with a graft labeled with iron oxide nanoparticles before transplantation. Iron-labeled islet cells underneath the kidney capsule appeared as pocket of signal loss disrupting contour of left kidney (arrow).
Reproduced with permission from the Radiological Society of North America [98].

SPIO probes conjugated with radioactive tracers has been developed [103].

In vivo imaging of stem-cell transplantation for diabetes

In T1D patients, the loss of only one cell type provides a unique opportunity as only this cell type and not a whole organ has to be generated or transplanted. In addition, the critical function of β-cells is to release insulin directly into the bloodstream, a function they can fulfill even when not placed into their original location. These aspects specific to diabetes suggest that patients suffering from T1D would be good candidates for stem-cell replacement strategies [104].

Stem cell-based therapies continue to show great promise for treatment of T1D, and significant progress has brought these therapies closer to a clinical reality through research efforts [105]. Pluripotent stem cells have the potential to differentiate into specialized cells of all three primary germ layers. Embryonic stem (ES) cells [106], induced pluripotent stem cells [107–109] and adipose tissue-derived stem cells [110–112] have to be tested for generating insulin producing cells that could potentially be used to treat T1D. Mesenchymal stromal cells, derived from bone marrow or other sources, have been utilized to improve engraftment of pancreatic islets by suppressing inflammatory damage and immune-mediated rejection [113–116].

For clinical translation, encouraging results were obtained in a small number of patients with early-onset disease who received autologous hematopoietic stem cell transplantation [117–121]. However, the results demonstrated that T1D patients responded differently to stem cell transplantation [119]. Therefore, *in vivo* imaging is urgently needed in this field for monitoring the survival and investigating the differentiation process of the transplanted stemss cells in real time. Recently, bioluminescence imaging (BLI) [122] was utilized for monitoring ES cell survival and differentiation into insulin-producing cells in a diabetic animal model. Raikwar *et al.* generated a double transgenic mouse ES cell line ectopically expressing Pdx1-*Aequorea coerulescens* GFP (AcGFP) fusion protein, and rat insulin promoter (RIP)-driven luciferase reporter. Real-time noninvasive BLI was used to monitor cell fate and function after transplantation. The authors speculated that pancreatic endoderm-like cells (PELCs) migrated into the STZ-damaged pancreas and differentiated into IPCs *in vivo* [123–125]. The differentiation of double transgenic ES cells transplanted under the renal capsule or systemically infused could be imaged by BLI as early as day 3 and until day 35 post-transplantation [123–125]. Considering that BLI is not an applicable clinical modality, MRI might be more advantageous for application in this field [23]. Magnetic nanoparticles represent a versatile platform suitable for theranostics imaging, precise drug-controlled release [126–128] and cell signaling control [36,129]. Similar to pancreatic islets, stem cells can be prelabeled with the above mentioned bifunctional nanoparticles for theranostic MRI. We are confident that theranostic MRI will play an important role for stem cell transplantation therapies for T1D in the future.

Conclusion

By combining diagnosis, therapy and targeting in one platform, theranostic imaging possesses the potential

to revolutionize the arena of healthcare [35]. Although there is an agreement on the potential that the theranostic holds, significant challenges remain and these key issues need to be addressed before clinical translation of these platforms. After systemic injection, nanoparticles will be taken up by reticuloendothelial system, in which probes are rapidly shuttled out of the circulation to nontarget organs such as liver, spleen and bone marrow. Therefore, the first challenge hampering theranostic MRI is that it is crucial to maximize the interaction of theranostic probes with the target tissues and to minimize the off-target uptake by other organs [16]. Another important issue is the difference in dose and circulation time between a therapeutic drug and an imaging agent. Imaging requires a higher signal for the area of interest compared with the surrounding tissue. Accordingly, most imaging probes need to be quickly cleared from the blood. However, a therapeutic drug usually is designed to have longer circulation times for adequate uptake by target tissues. We believe multimodality imaging platforms will play significant role in resolving these problems.

Future perspective

Although still in early phases of development, theranostic imaging represents promising new directions for clinical translation [16]. Considering the current developmental stage of theranostic MRI, it is still too early to predict its success; however, rapid advances in this field have promising potential for T1D management. Multifunctional theranostic imaging may indeed revolutionize drug delivery process and dramatically alter modern medicine in the future towards personalized medicine [16].

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Executive summary

Theranostic nanocarriers as smart actuators for MRI

- Imaging reagents used for theranostic MRI including iron oxide nanoparticles, gadolinium-loaded nanoparticles and manganese-based nanoparticles.
- Therapeutic and target moieties for theranostic MRI including siRNAs, DNA and miRNAs.

Type 1 diabetes pathological targets for theranostic MRI

- Imaging of vasculature changes during the progression of inflammation.
- Theranostic imaging of the lymphocyte infiltration during the progression of inflammation.
- Development of theranostic probes for targeting endogenous pancreatic β-cells.

Theranostic MRI in islet transplantation

- Theranostic MRI for graft rescue.
- Theranostic imaging of encapsulated islet grafts.
- Early detection of immune rejection in transplanted islets.

Outlook & perspective

- Multimodality theranostic imaging for Type 1 diabetes.
- *In vivo* imaging of stem cell transplantation for diabetes.

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- •• of considerable interest

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