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Brown Adipose Tissue as a Unique Niche for Islet Organoid Transplantation: Insights From In Vivo Imaging

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Background. Transplantation of human-induced pluripotent stem cell (hiPSC)-derived islet organoids is a promising cell replacement therapy for type 1 diabetes (T1D). It is important to improve the efficacy of islet organoids transplantation by identifying new transplantation sites with high vascularization and sufficient accommodation to support graft survival with a high capacity for oxygen delivery. **Methods.** A human-induced pluripotent stem cell line (hiPSCs-L1) was generated constitutively expressing luciferase. Luciferase-expressing hiPSCs were differentiated into islet organoids. The islet organoids were transplanted into the scapular brown adipose tissue (BAT) of nonobese diabetic/severe combined immunodeficiency disease (NOD/SCID) mice as the BAT group and under the left kidney capsule (KC) of NOD/SCID mice as a control group, respectively. Bioluminescence imaging (BLI) of the organoid grafts was performed on days 1, 7, 14, 28, 35, 42, 49, 56, and 63 post-transplantation. **Results.** BLI signals were detected in all recipients, including both the BAT and control groups. The BLI signal gradually decreased in both BAT and KC groups. However, the graft BLI signal intensity under the left KC decreased substantially faster than that of the BAT. Furthermore, our data show that islet organoids transplanted into streptozotocin-induced diabetic mice restored normoglycemia. Positron emission tomography/MRI verified that the islet organoids were transplanted at the intended location in these diabetic mice. Immunofluorescence staining revealed the presence of functional organoid grafts, as confirmed by insulin and glucagon staining. **Conclusions.** Our results demonstrate that BAT is a potentially desirable site for islet organoid transplantation for T1D therapy.

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Type 1 diabetes (T1D) is an autoimmune disease characterized by the destruction of insulin-producing β cells, which results in insulin deficiency, high blood glucose levels, and risk of a number of complications including cardiovascular disease, renal failure, and retinopathy.[1] The prevalence of T1D increases annually, with 64 000 individuals diagnosed annually in the United States. The first and most available treatment option for T1D is insulin therapy, which provides exogenous insulin either through multiple daily injections or insulin pumps, depending on the severity of the disease. Patients with T1D require lifelong exogenous insulin administration. However, because exogenous insulin cannot truly mimic the physiological insulin production and metabolism or clearance, fluctuations in blood glucose levels

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result in long-term complications associated with T1D.[2] To achieve normoglycemia, transplantation of pancreatic islets in patients with T1D offers a promising alternative option to injected insulin.[3,4] Nonetheless, there are several challenges associated with islet transplantation: scarcity of donor pancreas, insufficient oxygen and blood supply for the grafts, and immune rejection.[3,5] These challenges have hampered the widespread application of islet transplantation as a routine therapy for T1D patients. Generation of β cells or islet organoids from human-induced pluripotent stem cells (hiPSCs) reprogrammed from T1D patients' somatic cells may provide an alternative abundant source of β cells for transplantation into T1D patients. Several groups have differentiated hiPSCs into insulin-producing β-like cells or islet organoids in vitro. hiPSC-derived β-like cells or islet organoids have been shown to secrete insulin when transplanted into diabetic animals in vivo.[6-8] Recently, Shapiro et al[9,10] also demonstrated the feasibility and safety of using hiPSC-derived pancreatic endoderm cells to treat patients with T1D in clinical trials (ClinicalTrials.gov NCT03163511), highlighting the promise of a stem cell-based therapeutic approach. In addition, hiPSCs offer great hope that a personalized, "syngeneic" cell could be transplanted without the risk of alloimmunity, thereby providing sufficient supply to meet future global demand.[11]

The transplantation site microenvironment affects the survival and functionality of transplanted pancreatic islets or islet organoids.[12] The most widely used transplantation site in clinical practice is in the liver via intraportal islet infusion, but with a number of complications, such as loss of functional islet mass, bleeding and thrombosis, and progressive deterioration of intrahepatic islet function.[13,14] Identification of potential transplantation sites could improve the survival of islet and islet organoids and optimize graft function after transplantation.[2,15-17] More research on the optimal sites is needed to improve the success rate of the islet and islet organoid transplantation for long-term application. A key component in the successful optimization of islet graft survival after transplantation is the rapid establishment of blood flow for nutritional supply, oxygen supply, and immune regulation.[12,18] The ideal transplantation site should be vascularized, innervated, and immune-privileged, with an anti-inflammatory microenvironment and a low complication rate.

Brown adipose tissue (BAT) is a thermogenic tissue that generates heat in response to cold exposure and is characterized by a high degree of vascularization and sympathetic innervation.[19-22] Several studies have indicated that islets could be transplanted into BAT as a potential site to improve the outcomes of islet transplantation by delaying immune rejection, reducing adipose tissue inflammation, and without harming the BAT function.[23,24] The goal of this study was to determine whether transplanting human iPSC-derived islet organoids into the BAT in mice could improve graft survival compared with a widely used transplantation model of under kidney capsule (KC) for islet organoid transplantation.[25] We compare the outcomes using bioluminescence imaging (BLI) for these 2 groups (Figure 1).

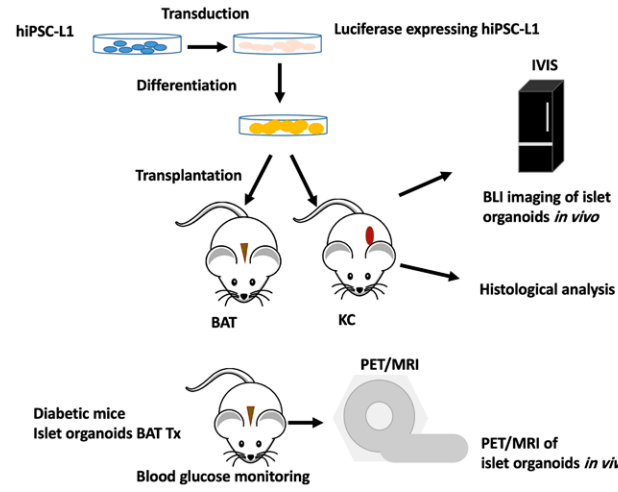

FIGURE 1. Flowchart of experimental design for this study. The goal of this study is to compare the outcomes of islet organoids transplantation into the BAT with the under the left KC models using in vivo BLI. BAT, brown adipose tissue; BLI, bioluminescence imaging; hiPSC, human-induced pluripotent stem cell; KC, kidney capsule.

MATERIALS AND METHODS

hiPSC-L1 Culture

Undifferentiated hiPSC-L1 was cultured in Essential 8 Flex medium (Thermo Fisher Scientific, Waltham, MA) containing 1% penicillin/streptomycin (Gibco, Billing, MT) on 6-well plates coated with growth factor-reduced Matrigel (Corning, Corning, NY) in an incubator at 37°C, 5% CO_2, until 60%–80% confluence was reached, at which point cells were split into new wells using ReLeSR passaging reagent as previously described[25,26] (STEMCELL Technologies, Vancouver, BC, Canada).

Lentiviral Vectors and Cell Transduction

The lentiviral vector (EFS-LUC2-Puro; VectorBuilder, Chicago, IL) was designed with an EFS promoter driving the expression of humanized luciferase (Luc2) and puromycin resistance for cell selection. EFS-LUC2-Puro Lentiviral particles were produced by triple-transient transfection of 3 plasmids (the transfer vector plasmid EFS-LUC2-Puro, packaging plasmid psPAX2, and envelope protein-coding plasmid pMD2.G) in 293T cells. The supernatants were collected, passed through a PVDF filter (SE1M003M00; MilliporeSigma, Burlington, MA), and purified by ultracentrifugation. Undifferentiated hiPSC-L1 cells were infected with lentivirus overnight with polybrene (TR-1003-G; MilliporeSigma). Puromycin was added and cells were maintained for positive cell selection.[26,27] Surviving clones were collected and expanded to establish the hiPSC-LUC2 cell line and replated for islet organoid differentiation.[26,27]

Pancreatic Islet Organoid Differentiation

To generate islet organoids, confluent cultures were dissociated into small cluster suspensions by incubation with Accutase (Innovative Cell Technologies, San Diego, CA). Cells (control hiPSC-L1 or luc expressing hiPSC-L1) were dispersed in 96-wells of low-adherence plates (Corning) containing 150 µL Essential 8 Flex Medium. The cells were differentiated into islet organoids according to the established protocols.[6,25] The differentiation medium formulations used were as follows. Stage 1 media: 500 mL MCDB 131 (CellGro, Lincoln, NE) supplemented with 0.22 g glucose (MilliporeSigma), 1.23 g sodium bicarbonate (MilliporeSigma), 10 g BSA (HyClone, Cytiva, Marlborough, MA, USA), 10 µL ITS-X (Invitrogen, Thermo

Fisher, Waltham, MA), 5 mL GlutaMAX (Invitrogen, Thermo Fisher), 22 mg vitamin C (MilliporeSigma), and 5 mL penicillin/streptomycin solution (CellGro). Stage 2 media: 500 mL MCDB 131 supplemented with 0.22 g glucose, 0.615 g sodium bicarbonate, 10 g BSA, 10 µL ITS-X, 5 mL GlutaMAX, 22 mg vitamin C, and 5 mL P/S. Stage 3 media: 500 mL MCDB 131 supplemented with 0.22 g glucose, 0.615 g sodium bicarbonate, 10 g BSA, 2.5 mL ITS-X, 5 mL GlutaMAX, 22 mg vitamin C, and 5 mL P/S. Stage 4 media: 500 mL MCDB 131 supplemented with 1.8 g glucose, 0.877 g sodium bicarbonate, 10 g BSA, 2.5 mL ITS-X, 5 mL GlutaMAX, 22 mg vitamin C, 5 mL P/S, and 5 mg heparin (MilliporeSigma). Stage 5 media were prepared by supplementing CMRL 1066 Supplemented (Mediatech, Manassas, VA) with 10% fetal bovine serum (HyClone, Cytiva) and 1% penicillin/streptomycin. After 5 stages of differentiation, islet organoids were tested for typical islet markers, including insulin and glucagon, using qPCR and immunofluorescence.

Glucose-stimulated Insulin Secretion Assay

Islet organoids (100 IEQ, aged between 28 and 31 d of differentiation, n = 2) were first preincubated in 1 mL of Krebs buffer (KRB) with low glucose concentration (2.8 mmol/L) and then rinsed twice with the same buffer to remove residual insulin. Subsequently, islet organoids were exposed to 1 mL of KRB with low glucose concentration (2.8 mmol/L) for 30 min in a cell culture incubator set at 37°C, after which the supernatants were collected. Following 2 additional washes with the low glucose KRB, the islet organoids were subjected to 30-min incubation in 1 mL of KRB with high glucose concentration (28 mmol/L), and the supernatants were collected thereafter. Human insulin levels were quantified using the Insulin Human ELISA kit (ab100578; Abcam, Waltham, MA). To normalize human insulin measurements, cell counts were obtained by dissociating the islet organoids into individual cells using Accutase (Innovative Cell Technologies), followed by counting with a cell counter.

Immunofluorescence Confocal Microscopy Imaging

Human islet organoids were transferred to microcentrifuge tubes (Eppendorf, Enfiled, CT) using a cut 1000-µL pipette tip to avoid disruption of the islet organoids and fixed in 4% paraformaldehyde solution for 8 min at 4°C. Fixation was followed by 3 washes in PBS and incubation in blocking solution (10% goat normal serum in PBS) on a thermal mixer (Thermo Scientific, Waltham, MA) at 300 RPM at 4°C overnight. Islet organoids were then incubated with primary antibody in the blocking solution (5% goat normal serum in PBS) on a thermal mixer at 300 RPM at 4°C for 24 h. Primary antibodies used are listed in Table 1. Primary antibody exposure was followed by 3 washes in PBS and incubation with the secondary antibody at room temperature for 1 h in the dark. The stained islet organoids were washed 3 times in PBS before moving into the container (microinsert 4 well dish, Ibidi) using mounting medium containing 4′,6-diamidino-2-phenylindole (Vectashield; Vector Laboratories, Newark, CA). Islet organoids were imaged using an Olympus FluoView 1000 filter-based laser scanning confocal microscope. Immunofluorescence images of islet organoids were semiquantitatively analyzed using the ImageJ2 (NIH, Bethesda,MD). Briefly, multicolor fluorescent images were split into single channels and converted to grayscale images. The area of interest was selected using selection tools from ImageJ2. The percentages of islet organoids that were positive for insulin and glucagon was calculated.

Mice

All animal experiments were performed in compliance with institutional guidelines and approved by the Institutional Animal Care and Use Committee at Michigan State University. Twelve-week-old female old NOD/SCID mice (Jackson Laboratory, Bar Harbor, ME) were housed on a 12 hours light/dark cycle at 22–23°C with ad libitum access to standard laboratory chow and acidified water. Diabetes was induced by intraperitoneal injection of streptozotocin (200 mg/kg body weight; Sigma-Aldrich) freshly dissolved in sodium citrate buffer.[28] Diabetes was confirmed by weight loss, polyuria, and blood glucose levels higher than 250 mg/dL (at least 2 tests).[28]

Islet Organoid Transplantation

Islet organoids were collected and transferred into a butterfly needle (25 gauge) connected with polyethylene tubing ID 0.76 mm (0.030″) OD 1.22 mm (0.048″) (INTRAMEDIC, PE60, Thermo Scientific) for both the KC group and the BAT group. Healthy and diabetic mice were anesthetized using 2% isoflurane. An incision was made to expose the left kidney of the mouse, the tubing was loaded with islet organoids (400 IEQ) inserted underneath the KC, and the islet organoids were transplanted underneath the KC as previously described.[25,29] Islet organoids BAT transplantation was performed via an interscapular incision. To expose the scapular white adipose tissue, it was cut and folded back to reveal the large bifurcated scapular BAT depot. An islet organoid (400 IEQ) suspension was infused into the left lobe of the BAT through polyethylene tubing attached to a butterfly needle. Mice from both groups were closely monitored posttransplantation.

Bioluminescence Imaging

Before anesthesia, mice were intraperitoneally injected with luciferin (PerkinElmer, Waltham, MA) at a dose of 150 mg/kg at a concentration of 15 mg/mL in calcium- and magnesium-free PBS as a substrate. Five minutes after luciferin injection, mice were sedated with a mixture of vaporized isoflurane and oxygen gas. Ten minutes after luciferin injection, the mice were imaged using IVIS SpectrumCT (PerkinElmer).[26] These 2 groups were followed up with BLI/CT on days 1, 7, 14, 28, 35, 42, 49, 56, and 63 posttransplantation. Quantitative

TABLE 1.

The list of the primary antibodies used for immunostaining

Primary antibody	Supplier	Catalog	Host	Target
Anti-insulin	Abcam	ab7842	Guinea pig	Mouse, rat, human, Syrian hamster
Anti-glucagon	Sigma	259A-1	Rabbit	Human
Anti-C peptide	Abcam	ab14181	Rabbit	Mouse, human

assessment of the BLI signal intensity of the grafts was performed using the Living Image software.

Positron Emission Tomography/MRI of Islet Organoids In Vivo

Positron emission tomography (PET)/MRI images were acquired using a Biospec 70 of 30 with a PET insert using Paravision 3.1 (Bruker BioSpin, Billerica, MA). Diabetic mice transplanted with 280 μg/mL VivoTrax (Magnetic Insight, Alameda, CA)-labeled islet organoids (400 IEQ, n = 2) and sham control mice without islet organoids were subjected to PET/MRI. Before imaging procedures, mice were fasted and kept in cold room (~4°C) for 4–6 hours. Mice were anesthetized with a 1%–2% isoflurane/O_2 gas mixture before imaging. A tail vein catheter was initially placed for injection of 100 μL of ^{18}F-fluorodeoxyglucose (FDG) tracer solution (0.15 MBq/g bodyweight). Animals were then kept in cold room (~4°C) for 40 min to increase FDG uptake in the BAT. Mice were positioned on top of a 4-cm 4-channel array receive coil and an 86-mm volume coil was used to transmit the RF signal. T2* weighted image: 2D T1 FLASH, TR/TE: 200/8 ms, field of view 30 × 30 mm, resolution 200 × 200 × 500 μm, 6 slices, flip angle 30°, 16 averages, and acquisition time 4 min 3 s. T2 weighted image: 2D-Turbo RARE, TR/TE: 2200/40 ms, RARE factor 8, field of view 30 × 30 mm, resolution 200 × 200 × 500 μm, 6 slices, 4 averages, acquisition time 2 min 38 s. T2map: multispin multiecho sequence, TR/TE: 2200/8.4, field of view 30 × 30 mm, resolution 200 × 200 × 500 μm, 12 echo images acquired (fewer points were used for generating maps, depending on the signal becoming equivalent to the noise), and acquisition time of 5 min 30 s. A 3D FISP image with a resolution of 200 × 200 × 400 μm was used for attenuation correction of the PET signal for the mouse images.

Mouse PET images were acquired for 30 min and reconstructed using a calibrated MLEM method with a resolution of 0.5 mm and 18 iterations, and corrections for scatter, randoms, decay, and partial volume.

Histology

The scapular fat pad was extracted, and the bifurcated BAT lobes were excised from the surrounding WAT. Islet organoids grafts of BAT were collected and processed for histologic analysis at 14-d posttransplantation as previously described.[25] The primary antibodies used are listed in Table 1. For quantifying insulin expressions in the islet organoid grafts, the area of interests (insulin positive) was selected using selection tool in ImageJ 1.46r software (NIH). The Corrected Total Cell Fluorescence (CTCF, the unit of measurement used by ImageJ to quantify specific levels of fluorescence) was calculated using the following formula: CTCF = integrated density – (area of selected cell × mean fluorescence of background readings).[30]

Statistical Analysis

Data are presented as the mean ± SD. Statistical comparisons between 2 groups were performed using Student t-test. Time course analysis was evaluated by the repeated 2-way ANOVA using GraphPad Prism 9 (GraphPad Software, La Jolla, CA). Statistical significance was set at $P < 0.05$.

RESULT

Characterization of Differentiated Islet Organoids

Human iPSC-L1 cells transduced with the vector of EFS-LUC2-Puro expressed stable bioluminescence signal as a "biomarker." After the addition of D-luciferin, a bioluminescent signal indicative of Luc2 activity was recorded at several passages after transduction.[26] Human iPSCs were differentiated into islet organoids in 3D-suspension culture using a stepwise protocol. Differentiated islet organoids expressed high level of islet cell markers of insulin and glucagon at both mRNA and protein levels. The expression of insulin and glucagon mRNAs in islet organoids were detected using quantitative RT-PCR (Figure 2A). Immunofluorescence imaging revealed the expression of insulin, glucagon, and C peptide in the differentiated islet organoids (Figure 2B, D). The static glucose-stimulated insulin secretion assay revealed that the differentiated islet organoids responded to glucose stimulation by secreting human insulin (Figure 2C).

BLI of Islet Organoids In Vitro and In Vivo

As shown in Figure 3A, islet organoids derived from luciferase-expressing iPSC-L1 cells displayed a bioluminescence signal (25 IEQ) after culturing with D-luciferin for 10 min (Figure 3A). No bioluminescence signal was observed in islet organoids derived from control iPSC-L1 (25 IEQ). After successful transplantation of islet organoids into the left lobe of BAT or KC of NOD/SCID mice, the BLI signals of islet organoids grafts were detected in all recipients from both the BAT (n = 4) (Figure 3B) and KC (n = 3) groups at the expected anatomic location on day 14 posttransplantation after injection of luciferin.

The bioluminescence signals measured using BLI on days 7, 14, 28, 35, 42, 49, 56, and 63 posttransplantation show gradual decreases in both BAT and KC groups (Figure 4). However, the graft BLI signal intensity under the left KC decreased substantially faster than that in the BAT ($P < 0.05$). In particular, on day 63 posttransplantation, there was no detectable BLI signal from the KC mice, whereas the graft BLI signal of the BAT group remained (Figure 4).

Islet Organoids Transplantation into the BAT Reverse Diabetes in Mice for Short Term

To evaluate the feasibility of BAT as a transplantation site in a diabetic mouse model, we transplanted islet organoids into the BAT of streptozotocin-treated mice (400 IEQ, n = 3). As shown in Figure 5, islet organoids transplantation into BAT restored euglycemia and maintained glucose for 1 week. In contrast, the mice in the control group that only received streptozotocin injection and sham surgery had hyperglycemia, which required insulin injection for treatment (0.75–1.25 IU/d, n = 3) (Figure 5).

In Vivo PET/MRI

To verify the successful transplantation of islet organoids in vivo, superparamagnetic iron oxide nanoparticle VivoTrax (Magnetic Insight)-labeled islet organoids were transplanted into the BAT in diabetic mice (n = 2). PET/MRI showed indeed that the islet organoids were transplanted in the intended location in these diabetic mice, which appeared as "black dots" in the BAT on T2*WI because of the presence of VivoTrax (Figure 6). ^{18}F FDG PET is an imaging technique that is most commonly used to assess BAT activity in rodents and humans.

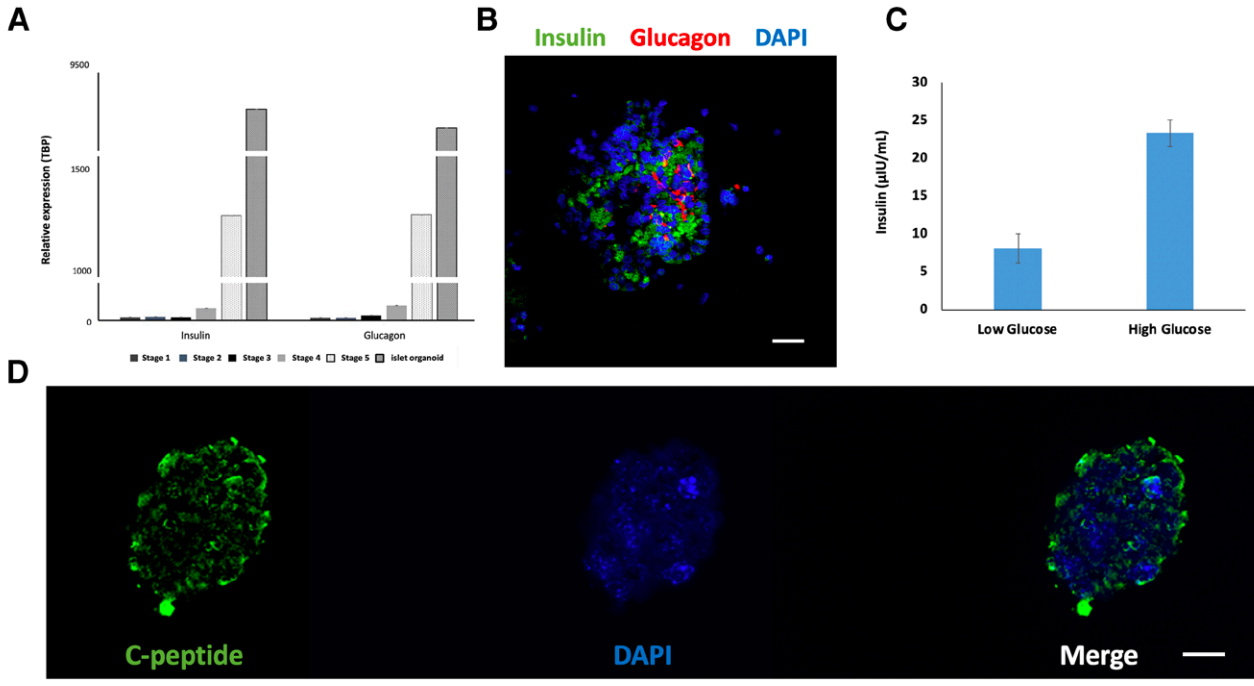

FIGURE 2. Differentiated islet organoids expressed islet cell markers of insulin and glucagon both at the RNA and protein levels. A, Quantitative reverse transcription polymerase chain reaction reverse transcription polymerase chain reaction analysis of insulin and glucagon mRNA expressions in the embryoid body at 5 stages under differentiation and in islet organoid. Results shown relative to the house keeping control TBP gene expression. Data are represented as mean ± SD. B, Immunofluorescence staining of differentiated islet organoids. Insulin (*green*, 32% ± 5%), glucagon (*red*, 27% ± 5%), and cell nuclei (4′,6-diamidino-2-phenylindole [DAPI], *blue*), magnification bar = 30 μm. C, ELISA measurement of secreted human insulin from islet organoids stimulated with low and high glucose, with a 30-min incubation for each concentration (n = 2). D, Immunofluorescence staining of C peptide (*green*) and cell nuclei DAPI (*blue*), magnification bar = 30 μm. TBP, TATA-box binding protein.

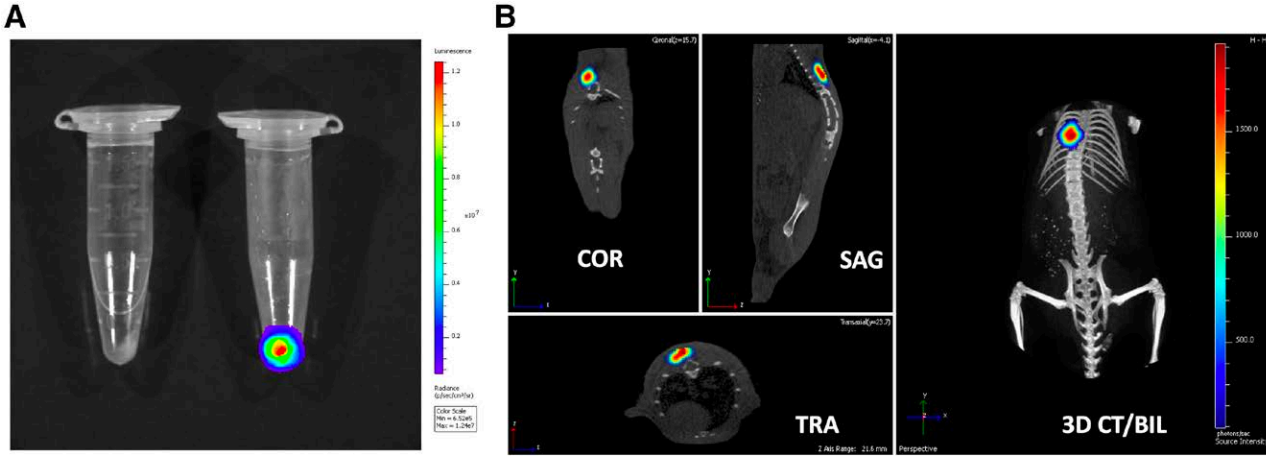

FIGURE 3. BLI of islet organoids in vitro and in vivo. A, BLI of islet organoids (25 IEQ) derived from control human-induced pluripotent stem cell (hiPSC-L1) without luciferase (*left*) and from luciferase expressing hiPSC-L1 (*right*). B, BLI reregistered with CT images of islet organoids graft in the BAT on day 14 posttransplantation (3D CT/BIL). BAT, brown adipose tissue; BLI, bioluminescence imaging; COR, coronal view; SAG, sagittal view; TRA, transverse view.

With cold treatment before in vivo imaging, the accumulation of ^{18}F FDG in the BAT was clearly demonstrated in these mice (Figure 6).

Ex Vivo Immunohistochemistry

Immunofluorescence staining of the BAT sections showed the presence of islet organoid grafts, as confirmed by double staining for insulin and glucagon 14-d posttransplantation (Figure 7), demonstrating islet organoids engraftment in BAT. Compared with double staining for insulin and glucagon of the KC sections, our data indicate that islet organoids in BAT yielded higher production of insulin than the KC group (Figure 7). In addition, the transplanted islet organoids appear to be scattered in a larger volume in BAT posttransplantation; these distribution patterns could also contribute to better oxygenation support to islet grafts in BAT. These ex vivo data are consistent with in vivo BLI results of reduction of BLI level by transplanted islet organoids in KC than in BAT (Figure 5), attesting to the feasibility of islet organoid transplantation in BAT.

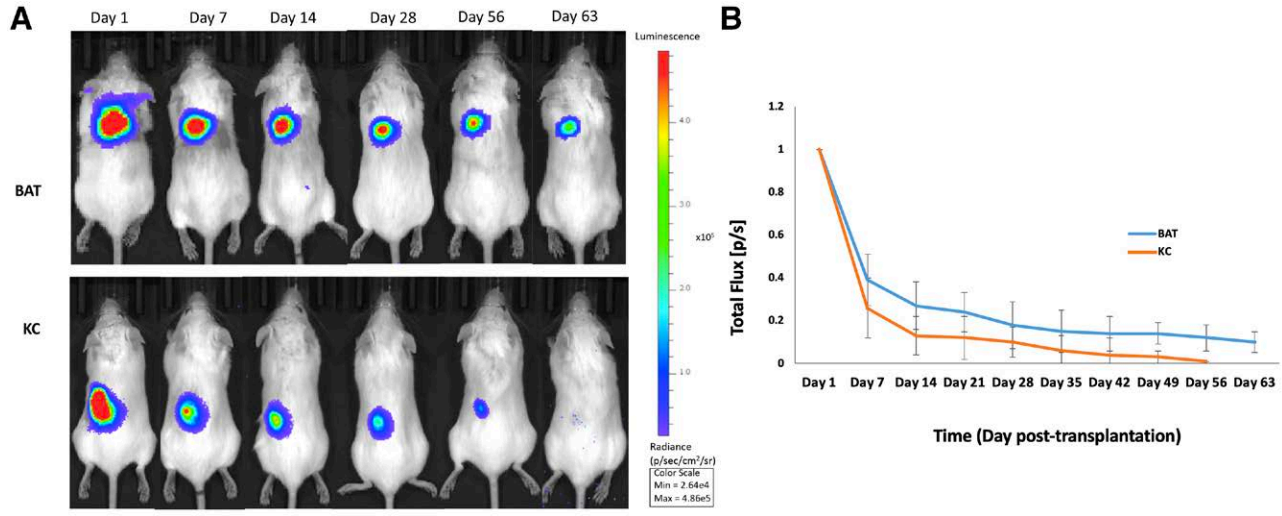

FIGURE 4. BLI signal comparison between BAT and KC groups. A, BLI follow-up of islet organoids (400 IEQ) derived from luciferase expressing human-induced pluripotent stem cell (hiPSC-L1) (*right*) transplanted in the BAT (*top*) and under the left KC (*bottom*). BAT, brown adipose tissue; BLI, bioluminescence imaging; KC, kidney capsule.

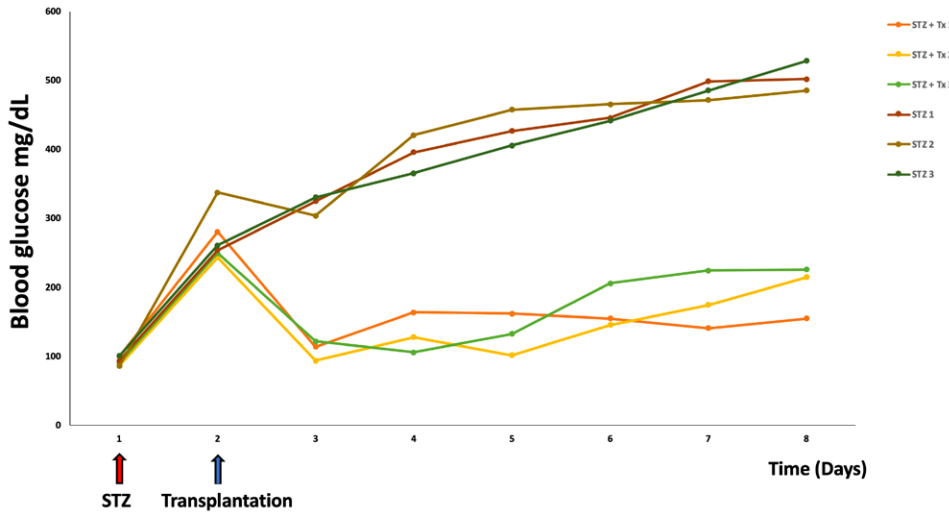

FIGURE 5. Islet organoids transplantation into BAT effectively restored euglycemia and maintained glucose blow 250 mg/dL of diabetic mice for a short-term up to a week (n = 3), compared with streptozotocin control group (n = 3). BAT, brown adipose tissue.

DISCUSSION

Transplantation of islet organoids derived from iPSC holds great promise for cell replacement therapy of T1D[9,10]; however, graft survival posttransplantation represents a bottleneck in the field of islet organoid transplantation. Cell replacement strategies have been performed in hepatic portal vein, KC, intramuscular, omentum, and subcutaneous sites, and have been performed in both animal models and human patients.[12,31,32] In mouse studies, the KC is the preferred site for islet or islet organoid transplantation,[12,25] because of its ease of surgical transplantation and graft retrievability. In humans, the most common site for islet transplantation is the hepatic portal vein in clinical practices, where engrafted islets could be damaged from instant blood-mediated inflammatory reaction (IBMIR) that is initiated by innate immune responses can cause substantial graft loss following intraportal transplantation.[33] For our current study, the decrease in bioluminescence signal over time following transplantation indicates acute islet organoid graft loss posttransplantation. Factors contributing to islet organoid loss include the lack of oxygen and blood supply-induced cell apoptosis, as well as the IBMIR, a nonspecific inflammatory and thrombotic reaction reported when allogeneic or xenogeneic islets come into contact with blood. We expected these signal changes on the basis of our previous work on superparamagnetic iron oxide nanoparticle-labeled islet organoid transplantation studies, wherein the labeled islet organoids were monitored using magnetic particle imaging (MPI) in vivo.[25] Interestingly, changes in MPI signals detected from islet organoids in KC mice corresponded with the bioluminescence signals in this current study.

iPSCs under current differentiation protocols for islet organoids progress toward 1 germ layer fate, the endoderm germ layer, and lose the ability to adopt alternative fates.[34] Islet organoid cells are derived from the endodermal germ layer, whereas endothelial cells (ECs) arise from the mesoderm

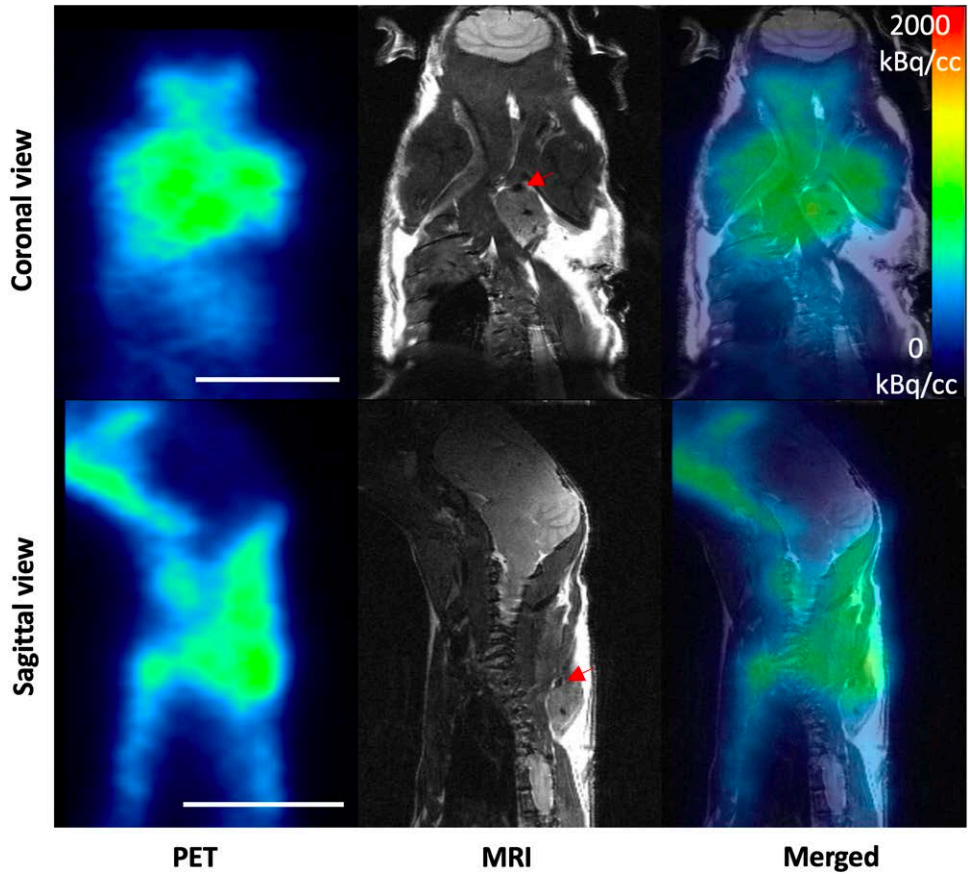

FIGURE 6. PET/MRI of islet organoids transplantation into BAT in diabetic mice. *Red arrows* show the VivoTrax-labeled islet organoids transplants in the BAT on T2*WI MRI. Co-registered PET images demonstrate accumulations of ^{18}F-fluorodeoxyglucose in the BAT transplantation site. *White line* is 1 cm. *Scale bar* of PET images is 0–2000 kBq/cc, same for both views. BAT, brown adipose tissue; PET, positron emission tomography.

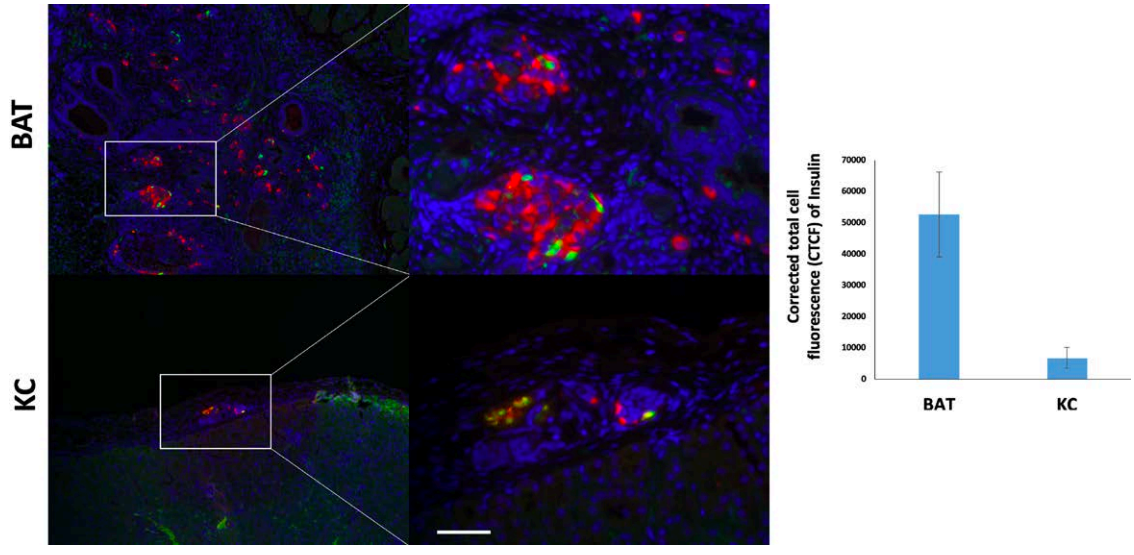

FIGURE 7. Double fluorescence immunostaining of islet organoid grafts in the BAT of insulin (*green*), glucagon (*red*), and cell nuclei (4′,6-diamidino-2-phenylindole [DAPI], *blue*) at 14-d posttransplantation, revealed the functional islet organoid graft in the BAT at 14-d posttransplantation (magnification bar = 30 μm). Double fluorescence immunostaining of islet organoid grafts in the KC showed significant graft loss after under kidney capsule transplantation of islet organoids. BAT, brown adipose tissue; KC, kidney capsule.

layer.[35,36] ECs cannot be generated during the process of islet organoids differentiation using current protocols.[35,37] It has been recognized that islet organoids require more densely vascularized tissue to support their survival and function compared with pancreatic islets[38,39] because ECs play a critical role in revascularization and support the function of grafts.

Interventions to improve the survival of islet organoid grafts and explore more suitable transplantation sites for islet organoids are urgently required.

This current proof-of-concept study demonstrates that the BAT is a favorable site for the transplantation of islet organoids. Unlike the portal vein, the dense peripheral vasculature and innervation in BAT allow not only survival with proper oxygenation and limiting sheer stress to the islet organoids,[40,41] but also for effective glucose response of beta-like cells.[38] Currently, there are clinical trials of transplantation of hiPSC-derived pancreatic endoderm cells that were assembled into vascularizing macroencapsulation devices to enhance cells survival, and were implanted subcutaneously in T1D patients.[9,10] A macroencapsulation device is a multilayer structure with a semipermeable membrane that has vascularizing portals and an external polyester mesh to provide the device structure. BAT is the optimal transplantation site for accommodating macroencapsulation devices loaded with islet organoids, and is easily accessible. BAT also contains niches of perivascular mesenchymal stem cells. They have the inherent capacity to secrete immunoregulatory, anti-inflammatory, and proangiogenic factors and, thus, have the potential to improve islet organoid engraftment, survival, and function.[23,41,42]

There are several limitations to our studies. These include small sample numbers, short-term follow-up of islet organoid function in the diabetic mouse model, a lack of functional measurements of the BAT to demonstrate the safety of this transplantation site. Additionally, we did not compare the functions of islet organoids between the diabetic BAT and KC animal models. We aimed to address these limitations in a larger animal study setting in the future. In summary, with this proof-of-concept study, we have demonstrated that the transplanted islet organoids are easily accessed and visualized by both BLI and PET/MRI, which is a clinically relevant imaging method.[43] These preliminary studies on transplanting hiPSC-derived islet organoids into the BAT provide promising evidence for the feasibility, safety, and efficacy of using the BAT as a transplantation site for stem cell-based therapies to treat T1D.

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