Subcutaneous transplantation of embryonic pancreas for correction of type 1 diabetes

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Subcutaneous transplantation of embryonic pancreas for correction of type 1 diabetes. Am J Physiol Endocrinol Metab 296: E323–E332, 2009. First published December 9, 2008; doi:10.1152/ajpendo.90544.2008.—Islet transplantation is a promising therapeutic approach for type 1 diabetes. However, current success rates are low due to progressive graft failure in the long term and inability to monitor graft development in vivo. Other limitations include the necessity of initial invasive surgery and continued immunosuppressive therapy. We report an alternative transplantation strategy with the potential to overcome these problems. This technique involves transplantation of embryonic pancreatic tissue into recipients' subcutaneous space, eliminating the need for invasive surgery and associated risks. Current results in mouse models of type 1 diabetes show that embryonic pancreatic transplants in the subcutaneous space can normalize blood glucose homeostasis and achieve extensive endocrine differentiation and vascularization. Furthermore, modern imaging techniques such as two-photon excitation microscopy (TPEM) can be employed to monitor transplants through the intact skin in a completely noninvasive manner. Thus, this strategy is a convenient alternative to islet transplantation in diabetic mice and has the potential to be translated to human clinical applications with appropriate modifications.

Therapies for type 1 diabetes; embryonic pancreatic tissue; subcutaneous site; stem cell-derived islet tissue

Type 1 diabetes is a serious disease that affects about two million Americans, causing life-threatening complications in over 100,000 patients per year (32). Patients with type 1 diabetes require lifelong insulin replacement therapy administered via daily injections or insulin pump. Not only is this inconvenient, but intensive exogenous insulin therapy could lead to complications from hypoglycemia unawareness. A promising approach for more permanent and long-term insulin replacement is islet transplantation (50, 53). However, this approach has numerous limitations, such as the need for millions of donor islets, need for initial invasive surgery and prolonged immunosuppressive therapy, poor graft survival, and lack of suitable techniques to monitor transplant development in vivo. Such problems greatly limit the long-term success rate of islet transplantation (3, 6–8, 14, 50, 53). The ideal transplantation strategy for type 1 diabetes should include minimally invasive placement of transplants, no need for immunosuppression, long-term reversal of diabetes, and the possibility for noninvasive in vivo monitoring of transplants. We report considerable success in achieving these goals in diabetic mouse models through subcutaneous transplantation of GFP-expressing embryonic pancreatic tissue into recipient mice with type 1 diabetes followed by noninvasive graft monitoring with two-photon excitation microscopy (TPEM).

Even though the techniques for intrahepatic embolization of islets through portal vein are being progressively refined, life-threatening complications, such as portal hemorrhage, portal hypertension, and thrombosis, can still occur (3, 6, 9, 50). The subcutaneous space is a superficial and easily accessible site where transplants can be performed in a minimally invasive manner with no risk and little stress to recipients. In addition to eliminating risks associated with portal vein cannulation, the subcutaneous site has the added advantage of extensive surface area and the option for repeated transplants if necessary. However, this site is limited by poor vascularization potential, resulting in low success rates in previous attempts at islet transplantation (19, 28, 54, 63). Although different techniques are being developed to improve graft vascularization in the subcutaneous space, these require complex manipulations and/or multiple surgeries for the same transplant (10, 29, 42, 59, 63). Transplantation of embryonic tissue as opposed to mature islets is a simpler solution for maintaining graft survival and vascularization in the subcutaneous space. Being a built-in source of angiogenic factors, embryonic tissue may achieve better vascularization than mature islets (44–46). It is possible that embryonic tissue may also resist rejection due to its immune-privileged nature (16–18, 26, 31, 34, 41), although the immunogenicity of embryonic pancreatic tissue has not yet been demonstrated. Embryonic pancreas from mice expressing green fluorescent protein (GFP) under the regulation of insulin 1 promoter (MIP-GFP mice) emit easily detectable bright fluorescence from their β-cells after embryonic day E13.5 (20, 21), providing an excellent model for in vivo graft monitoring through the intact skin.

The current study demonstrates successful endocrine differentiation, vascularization, and significant improvement of blood glucose homeostasis following subcutaneous transplantation of embryonic pancreata into immune-deficient diabetic mice. In addition to normalization of glucose tolerance, subcutaneous transplants also enabled diabetic mice to gain back the lost adipose tissue and enhanced overall well-being. Considering that these outcomes occurred without specialized maneuvers, it is predictable that better graft survival rates in non-immune-deficient animals may be achieved through simple facilitation techniques such as addition of exogenous growth factors or anti-inflammatory agents. Thus, the current results demonstrate a successful first step toward correction of type 1 diabetes through noninvasive transplantation, which can be refined and customized for human patients through appropriate modifications such as the use of stem cell-derived islet tissue.

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over the kidney, and the kidney was exposed by gently pressing below

anlegesia was provided with buprenorphine 0.1 mg
sia with ketamine-xylazine (110/10 mg/kg ip), and postoperative
available pancreata. Surgeries were performed under general anesthe-
ata were transplanted at each site, depending on the size of the
transplants without pancreatic tissue. Two to four embryonic pancre-

METHODS

Animals

Donor embryonic pancreata were obtained from MIP-GFP mice, originally donated by Dr. Graeme Bell at the University of Chicago and now maintained in our colony (20, 21). Recipients were immune-
deficient NCRNU-M-M nude mice (Taconic) (60), and NOD-SC-
M mice (Taconic) (61) rendered diabetic with streptozotocin (125 mg/kg ip, repeated every 2 wk as necessary until diabetes induced). All recipients were males aged 2–4 mo. Animals were fed standard laboratory chow and were cared for according to the guidelines of the Vanderbilt Institutional Animal Care and Use Committee.

Isolation of Embryonic Pancreata

Pregnant females carrying MIP-GFP embryos (E14.5-E16.5) were used. Gestational age E14.5-E16.5 was selected by comparing the

Table 1. Changes of body weight before and 3 mo after transplants in each group

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal NCRNU mice</td>
<td>27.6±1.04</td>
</tr>
<tr>
<td>Diabetic pretransplant</td>
<td>25.6±0.82</td>
</tr>
<tr>
<td>Subcutaneous transplants</td>
<td>28.23±1.07*</td>
</tr>
<tr>
<td>Renal subcapsular transplants</td>
<td>26.35±0.35</td>
</tr>
<tr>
<td>Untreated diabetic controls</td>
<td>22.2±0.74</td>
</tr>
</tbody>
</table>

*P < 0.05 when subcutaneous transplant group was compared with diabetic pretransplant conditions and/or untreated diabetic controls.

Transplant Monitoring

Anesthetized mice were placed on a customized stage on the LSM510 META confocal microscope (Zeiss). The approximate area of GFP fluorescence was first detected with confocal microscopy (excitation 488 nm, emission 500- to 550-nm bandpass filter). Deeper and more detailed images of the transplant were then taken with TPEM using a Coherent Chameleon laser (excitation 900 nm, emission 500–550 nm). To distinguish the transplants from nonspecific fluorescence emitting from autofluorescent structures, the emission spectrum of each fluorescent structure was measured with narrow spectral bands by use of the META detector of a Zeiss LSM510. These emission spectra were then compared with the emission spectra of true GFP in isolated islets from MIP-GFP mice.

Histological Studies

Mice were killed 3–5 mo after transplantation, and the transplants were excised. Excised tissue samples were fixed in 4% paraformal-dehyde for 2 h and washed several times in 1× phosphate-buffered saline in preparation for histological sectioning. Sections (5 µm) of transplants were analyzed by immunostaining for insulin and CD34 (counterstained with Alexa fluor 488 and Alexa fluor 568, respectively) to verify differentiation of β-cells and vascular endothelial cells. Other sections were stained for glucagon for further verification of endocrine differentiation and with bromodeoxyuridine (BrDU) to check for neoplastic transformation (both secondary stained with Alexa fluor 568 in separate sections). Sectioning and staining were performed at the Vanderbilt Immunohistochemistry Core Facility.

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Fig. 2. Subcutaneous transplants normalize glucose tolerance in streptozotocin-treated NCRNU nude mice. TP, transplant; SubQ, subcutaneous; Renal sub, renal subcapsular; DBS, dorsal body surface. IPGTT was performed under different conditions, as noted, by injection of sterile glucose (2 g/kg ip) and blood collection at denoted time points under isoflurane-oxygen. Streptozotocin treatment resulted in severe impairment of glucose tolerance (●-solid line, n = 11) with drastic deviation from normal situation (●-solid line, n = 11). The renal subcapsular transplant group (positive control) brought glucose tolerance back to normal (●-dashed line, n = 4), whereas the untreated diabetic control group, which did not receive pancreatic transplants (negative control), continued to show progressive impairment of glucose tolerance (●-dashed line, n = 6; *P < 0.05 at every time point except 30 min), with a 50% success rate (3 of 6). A: subcutaneous transplants of embryonic days E15.5–E16.5 embryonic pancreas placed in the earlobe site produced significant improvement of glucose tolerance compared with negative controls (●-dashed line, n = 6; *P < 0.05 at every time point except 30 min), with a 50% success rate (3 of 6). B: subcutaneous transplants placed in the DBS (●-dashed line, n = 5) produced complete normalization of glucose tolerance similar to normal nondiabetic group or renal subcapsular transplant group. Excision of subcutaneous transplants also resulted in progressive impairment of glucose tolerance (●-dotted line, n = 3). Changes in glucose tolerance were first observed at 3 wk posttransplant and have been followed up to 6 mo so far. Representative data at 12 wk posttransplant placement are shown here. **P < 0.001 for each time point vs. corresponding time point of any negative control.

Statistical Analysis

Values are expressed as means ± SE; n denotes the number of animals in each group. Groups were compared using paired Student’s t-test.
FIGURE 4. Subcutaneous transplants improve glucose tolerance and insulin response to glucose challenge in streptozotocin-treated NOD-SC mice. IPGTT was performed by injection of sterile glucose (2 g/kg ip) and blood collection at denoted time points under isoflurane-oxygen. A: glucose tolerance. Streptozotocin treatment resulted in severe impairment of glucose tolerance ( ). Significant improvement of glucose tolerance resulted from subcutaneous transplants of E14.5 embryonic pancreas in the earlobe site ( ). Representative data at 12 wk posttransplant placement are shown here. * \( P < 0.05 \) when average blood glucose levels at each time point in the subcutaneous transplant group was compared with corresponding time points of the untreated diabetic group. B: corresponding insulin response to glucose challenge during IPGTT in A. Insulin response is impaired in untreated controls (open bars) and shows some improvement in the subcapsular transplant group (filled bars). Significant increase of insulin response seen at the 2-h time point in the subcutaneous transplant group. * \( P < 0.05 \) vs. corresponding time point in the diabetic control group.

RESULTS

Normal body weight of NCRNU nude mice (8–16 wk of age) ranged from 25 to 30 g, averaging 27.6 ± 1.04 g. Treatment with streptozotocin resulted in weight loss of up to 10% of body weight and development of other clinical symptoms such as polyuria, polydipsia, and polyphagia. As shown in Table 1 and Fig. 1, subcutaneous transplants reversed streptozotocin-induced weight loss, exceeding prediabetic body weight. Clinical observations indicated reduced symptoms of diabetes in the transplant group. As expected, the renal subcapsular transplant group also regained the adipose tissue lost due to streptozotocin treatment, although they did not exceed prediabetic weight (Table 1). The untreated diabetic control group, which did not receive pancreatic transplants, continued to lose weight, up to 20% of body weight, and exhibited worsening symptoms until the time of euthanasia.

Figure 2 shows the effects of transplants on glucose tolerance in NCRNU nude mice. Transplants were performed on streptozotocin-treated mice that showed fasting glucose levels over 300 mg/dl. In some animals, diabetes was not induced by a single injection of streptozotocin, and two or three biweekly injections were necessary. Once induced, diabetes was persistent and progressive, causing severe impairment of glucose tolerance (Fig. 2). As expected, renal subcapsular transplants produced a significant improvement of glucose tolerance, bringing it back to normal. Subcutaneous transplants were first placed in the earlobe, as this was a better site for parallel imaging studies. Fifty percent of the earlobe transplants were successful in normalizing glucose tolerance, whereas the remaining 50% were unsuccessful (Fig. 2A). In contrast, all of the subcutaneous transplants placed in the dorsal body surface produced complete reversal of diabetes, with a 100% success rate at 3 mo after transplantation (Fig. 2B), which lasted until the time of euthanasia at 5 or 6 mo posttransplant. The performance of successful subcutaneous transplants was comparable to those of normal nondiabetic mice or renal subcapsular transplants, bringing glucose tolerance back to normal.
explanation for the unusual insulin data is the overall weak insulin response characteristic to this mouse strain (Fig. 3B). Normal nondiabetic NCRNU nude mice maintain glucose tolerance comparable to wild type mice (Fig. 3B). However, their basal insulin levels are rather low compared those of with wild-type mice, and, unlike with wild-type mice, their plasma insulin level does not rise until 1 h after glucose challenge (albeit to a small degree) (Fig. 3B). Thus, it appears that the NCRNU strain can maintain normal glucose tolerance with considerably low plasma insulin levels, presumably through
SUBCUTANEOUS TRANSPLANTATION OF EMBRYONIC PANCREAS

A

Control Pancreas

SubQ Transplant

B

SubQ Transplant

C

Control Pancreas

SubQ Transplant
high sensitivity to insulin in peripheral tissues. Recent studies show evidence of specific adipokines that enhance insulin sensitivity in the peripheral tissues in the absence of increased levels of insulin (24). Furthermore, considering that the subcutaneous transplants enable a robust recovery of lost adipose tissue, it is likely that adipokines may contribute to the improved glucose homeostasis in addition to insulin in the subcutaneous transplant group. We provide more evidence for this possibility in subsequent sections.

In streptozotocin-treated NOD-SC mice, both IPGTT and insulin responses were significantly improved following subcutaneous transplants placed in the earlobes (Fig. 4). Endocrine differentiation of subcutaneous transplants was monitored non-invasively through the intact skin by use of TPEM. Although TPEM is an excellent method for monitoring transplant development in live animals, having to image through several layers of tissue reduces the clarity and resolution of images compared to imaging in vitro. Subcutaneous transplants in the earlobes are covered by epidermal and connective tissue at a depth of 100–150 μm. These were detectable by TPEM, whereas transplants in the dorsal body surface were undetectable so far due to excessive thickness of adipose tissue. During the first few weeks following placement, earlobe transplants could be easily located by their GFP fluorescence, which progressively spread out into the surrounding tissue (Fig. 5A), indicating transplant growth and expansion. One difficulty encountered in transplant monitoring was the presence of autofluorescent structures with high brightness, such as hair follicles. True GFP fluorescence emitted from transplants can be distinguished from autofluorescence by measuring the emission spectrum of each fluorescent area with a spectral detector (Fig. 5B). We know that that GFP emits light from 500 to 550 nm, with peak emission at 514 nm. Autofluorescent structures also emitted light in the range of 500–550 nm (and beyond), so were indistinguishable from GFP. To distinguish GFP from autofluorescent structures, we measured the peak emission of each fluorescent structure within the range of 500–550 nm. GFP had peak emission between 510–520 nm, whereas other structures had peak emission elsewhere (~540 nm in this case) (Fig. 5B). These results were later confirmed by immunostaining for insulin.

Transplant vascularization was verified by simultaneous immunostaining for insulin and CD34, an endothelial marker. Unlike the earlobe transplants, the transplants placed under the skin of the dorsal body surface were detectable by naked eye (Fig. 1) and could be removed as a whole for immunohistochemistry (Fig. 6). The transplants excised after 4 mo contained well-formed islets similar to those in control pancreas, as well as scattered, separate, insulin-staining cells. Islets in transplants ranged from 25 to 200 μm in diameter, comparable to control pancreatic islets. The excised transplants showed extensive neovascularization as indicated by CD34 staining (Fig. 6), comparable to control islets from normal pancreas. Improved vascularization was observed when the transplanted embryonic pancreata were from day E15.5 or later and when transplants were excised at 4 mo or later. Endocrine differentiation was further confirmed by the presence of glucagon-containing cells (Fig. 6B). No neoplastic transformation was detected, as indicated by BrDU staining (Fig. 6C), which was similar to control pancreas.

Since the normalization of glucose tolerance in the subcutaneous transplant group of NCRNU mice was not accompanied by a corresponding increase of plasma insulin, we performed additional experiments to explore a possible role in adipokines in glucose control. Plasma adiponectin levels were compared in the prediabetic, diabetic, and posttransplant groups. As Fig. 7 shows, streptozotocin-treated diabetic mice had significantly lower adiponectin levels compared with normal nondiabetic mice, and these levels did not increase following a glucose challenge. The subcutaneous transplant group had significantly higher adiponectin levels, which, unlike in the normal group, continued to increase in response to the glucose challenge. Thus, it appears that adiponectin plays an active role in maintaining glucose homeostasis following subcutaneous placement of embryonic tissue.

Fig. 6. Endocrine differentiation and neovascularization of transplants verified by immunohistochemistry. A: control pancreas showing islets (left 2 panels) and different areas of subcutaneous (SubQ) transplant on DBS of NCRNU nude mouse (E16.5, excised after 4 mo; right 4 panels); immunostained for insulin (green, counterstain Alexa fluor 488) and CD34 (red, counterstain Alexa fluor 568). Transplant has differentiated into distinguishable islets similar to those in control pancreas. Vascularization of islets is indicated by CD34 staining inside islets. Large blood vessels are seen in vicinity of islets. Dimensions of structures are as follows. Islets in control pancreas: 250 × 160 μm; top; 200 × 90 μm; bottom. Islets in SubQ transplant: top left 90 × 180 and 125 × 80 μm; top right 125 × 90 μm; bottom left 80 × 60, 55 × 75, and 65 × 45 μm; bottom right 50 × 50, 25 × 25, and 40 × 40 μm. Large blood vessels (white vertical arrows) around islets: control pancreas 40–60 μm in diameter; SubQ transplant 60–200 μm in diameter. Small blood vessels inside islets (blue horizontal arrows): 1–12 μm in diameter in both control pancreas and SubQ transplant. B: glucagon staining in islets of subcutaneous transplant, indicating further endocrine differentiation. Green, GFP fluorescence in β-cells; red, glucagon, secondary stain with Alexa fluor 568. Islet diameter: 50, 80, and 60 μm in left; 80 × 65 μm in right. C: minimal bromodeoxyuridine (BrDU) staining in islets of control pancreas and subcutaneous transplant, indicating absence of neoplastic transformation. Green, insulin, secondary stain with Alexa fluor 488; red, BrDU, secondary stain with Alexa fluor 568. Islet diameter: 150 and 100 μm in control pancreas, 100 and 200 μm in subcutaneous transplant.
DISCUSSION

Although islet transplantation is a commonly used treatment for type 1 diabetes with great success in the short term, up to 90% of patients are reported to revert to insulin dependence within 5 years (50). The success rate also varies widely with the quality of available donor tissue as well as the experience of the center performing the transplant (53). Portal vein cannulation, a requirement for traditional islet transplantation, carries the risk of life-threatening complications from hemorrhage, thrombosis, and portal hypertension (3, 6, 9, 53). Transplantation at a superficial and more accessible site such as the subcutaneous space would eliminate surgical complications, minimize stress associated with transplant placement, and enable better graft monitoring and addressing potential problems in the long term. However, previous attempts at islet transplantation at the subcutaneous site have not been very successful (19, 28, 54, 63) and/or required specialized manipulations (10, 29, 42, 59, 63). A recent study reported successful reversal of diabetes and minimally invasive graft monitoring with islet transplantation in the anterior chamber of the mouse eye (55). Although this compartment could become an excellent research model with demonstrated success, the eye is not a suitable therapeutic transplant site in a clinical setting, and repeated imaging carries the potential for tissue damage. Thus, the need remains for noninvasive transplant strategies that are customizable for clinical situations.

We have shown that an alternative technique for minimally invasive transplantation with potential long-term success is to use embryonic pancreatic tissue in the subcutaneous space. This superficial site offers many advantages such as no-risk transplant placement, noninvasive imaging, and ability to perform repeated transplants if necessary. In addition, the use of embryonic tissue eliminates the need for large numbers of donor islets and can be customized for human patients by using stem cell-derived islet-like cell clusters (27, 30, 33, 52, 56). In the current experiments with immune-deficient nude mice and NOD-SC mice, embryonic pancreata survived in the subcutaneous space with no additional facilitation techniques and produced remarkable improvement of glucose homeostasis and body weight. The possibility of false positive results due to potential spontaneous reversal of streptozotocin-induced diabetes was eliminated by excision of some transplants as well as maintaining an untreated diabetic control group for comparison.

An interesting finding was that the improvement of glucose tolerance occurred without a significant increase in plasma insulin response. Although this is somewhat unusual, NCRNU nude mice appear to have higher sensitivity to insulin, enabling them to maintain normal glucose tolerance with lower insulin levels. Although there was no information on this strain in the literature, our data show that they maintain normal glucose tolerance with considerably low insulin levels in their normal nondiabetic status (Fig. 3B). This may be a possible effect of higher insulin sensitivity at the peripheral tissue level unique to this strain, that merits further investigation. As several recent studies have reported, hormones from adipose tissue play an important role in enabling the mice in the current study to function without a detectable increase in insulin.

As Fig. 1 and Table 1 show, the subcutaneous transplant group showed increased body weight, gaining back all the adipose tissue lost following streptozotocin treatment and surpassing the pre-streptozotocin weight. Although successful treatment of type 1 diabetes is generally associated with recovery of body weight, there was also a significant and clinically visible increase in the adipose tissue in our subcutaneous transplant group compared with the renal subcapsular transplant group. Thus, it is possible that the glucose-lowering effect was partially independent of insulin and produced by adiponectin (or other adipokines) from the adipose tissue instead. To verify this, we tested plasma adiponectin levels in nondiabetic, diabetic, and transplant groups.

As Fig. 7 shows, streptozotocin-treated diabetic mice have significantly lower adiponectin levels compared with normal, nondiabetic mice, and these levels do not increase following a glucose challenge. The subcutaneous implant group has significantly higher adiponectin levels, which, unlike in the normal group, continue to increase in response to the glucose challenge. Thus, it appears that adiponectin plays an active role in maintaining glucose homeostasis following subcutaneous placement of embryonic tissue. This is not surprising, since adiponectin as well as several other adipokines are reported to exert strong effects on plasma glucose homeostasis. While some adipokines, such as resistin and retinol-binding protein-4, tend to exacerbate hyperglycemia (11, 23, 51, 58), a number of adipokines exert beneficial properties in glucose homeostasis, either directly decreasing blood glucose or enhancing sensitivity to insulin (4, 11, 23–25, 64–66). Particularly important among these are adiponectin, visfatin, and leptin. Visfatin is reported to exert insulin-like properties and lower blood glucose levels by binding to insulin receptors (25). Leptin was previously reported to improve glucose homeostasis through downregulation of resistin (4), while a recent study shows that leptin administration alone can lead to reversal of type 1 diabetes without insulin replacement (66). Adiponectin, whose levels decrease in diabetes (11, 64), has been known to increase insulin sensitivity in peripheral tissues in the absence of an increase of plasma insulin levels (24). Thus, adiponectin is a likely candidate that contributes to maintaining glucose homeostasis. As Fig. 7 shows, streptozotocin-treated diabetic mice have significantly lower adiponectin levels compared with normal, nondiabetic mice, and these levels do not increase following a glucose challenge. 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nonimmune suppressed diabetic rats and rhesus monkeys. Studies by Dafoe’s group (1, 2, 13, 57) reported success in transplantation of fetal pancreas in relatively superficial sites such as muscle with the aid of growth factors or simultaneous transplantation of fetal liver tissue. Our study confirms the value of embryonic pancreatic tissue as a superior source of insulin-producing cells and demonstrates the potential advantages of the subcutaneous space as opposed to traditional, deeper sites for transplantation. Another important advantage of placing transplants in the subcutaneous space is the ability to monitor transplant survival and development in a noninvasive manner. Monitoring transplants in traditional sites such as the omentum and renal subcapsular space require techniques such as skin fold chamber preparations or repeated surgery (35–39, 62), which are traumatic to the subject, inconvenient to the researcher, and unfeasible in a clinical setting. Unlike the traditional deeper transplants, subcutaneous transplants can be monitored noninvasively through the intact skin with modern imaging techniques (5, 12, 15, 22, 40, 43). We have used TPEM to monitor endocrine differentiation through GFP expression. Aided by histological verification (Fig. 6), we are in the process of optimizing TPEM parameters for progressive imaging of transplant vascularization in vivo.

Considering the positive results obtained without specialized manipulations, this study demonstrates the potential therapeutic value of subcutaneous transplantation of embryonic pancreatic tissue as a convenient alternative to traditional islet transplantation in deeper sites and points to an intriguing alternative mechanism of glycemic regulation through adipokines in addition to or instead of insulin. Our future directions include a thorough examination of such alternative glycemic control through measurements of different adipokines in normal, diabetic, and transplant conditions; exploration of possible structural and functional changes produced in adipose tissue by transplants; and verifying whether type 1 diabetes can be corrected through administration of specific adipokines or transplantation of adipose tissue alone. The current success of subcutaneous transplants with immune-deficient mouse strains is encouraging, and we hope to verify whether similar results can be achieved in nonimmunedeficient animals with or without facilitation techniques such as adding exogenous growth factors or anti-inflammatory compounds, culturing embryonic pancreas in different media prior to transplantation, and/or using specific gestational ages optimal for immune tolerance. Long-term goals are to customize this strategy for humans and companion animals by using stem cell-derived islet tissue.

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